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The Journal of Allergy

VOL. 6 NOVEMBER, 1934 No. 1

Original Articles

A SPECIES NONSPECIFIC ANTIGENIC FACTOR IN MAMMALIAN SERUMS*†

A PRELIMINARY REPORT

FRANK A. SIMON, M.D.
BOSTON, MASS.

AS ONE experiment in a study of the development of hypersensitiveness in man, a group of patients with various allergic diseases was injected intracutaneously with 0.10 c.c. of a 1:100 dilution of guinea pig serum.[1] In one patient, H. D., a man of twenty-seven years with chronic vasomotor rhinitis, the very first injection of guinea pig serum resulted in a severe general reaction with massive swelling of his face and hands and with generalized urticaria, but no asthma. Some days later, the degree of his sensitiveness was tested by injecting high dilutions of serum, and H. D. was found to react both to horse serum and to guinea pig serum in dilutions as high as one to ten million. Other tests showed strong reactions to extracts of the dander of the horse, cat, rabbit, and guinea pig.

How could these findings be explained? The man H. D. was an American musician. As a boy, he had had some contact with horses, but he denied ever having an injection of horse serum, even a dose of the diphtheria toxin-antitoxin mixture. He did not remember ever seeing a guinea pig. The possibility of a common, heterophile antigen was considered, and to throw light on this, a number of experiments were carried out.

First, H. D. was tested by the scratch method with the serum from a considerable number of different animals. This number included not only the common domestic animals, but thanks to the cooperation of the Curator of the Franklin Park Zoo, the serum of an elephant was ob-

*From the Medical Laboratories, Massachusetts General Hospital, Boston, Mass.

†Aided by a grant known as the M. G. H. Asthma Fund.

Read before the Twelfth Annual Meeting of the Association for the Study of Allergy, Cleveland, O., June 11, 1934.

tained, and Dr. Wislocki of the Department of Anatomy kindly provided samples of serums from an opossum and from a South American monkey. All these serums were diluted with saline containing 0.5 per cent of carbolic acid and used for tests by the scratch method. The results are shown in Table I.

In the original notes, all the reactions are recorded by facsimile drawings of the actual wheals and surrounding erythema. Fig. 1 is a sample and shows the sizes of reactions obtained with the serums of the cat, elephant, opossum, and South American monkey. Figures represent diameters in millimeters.

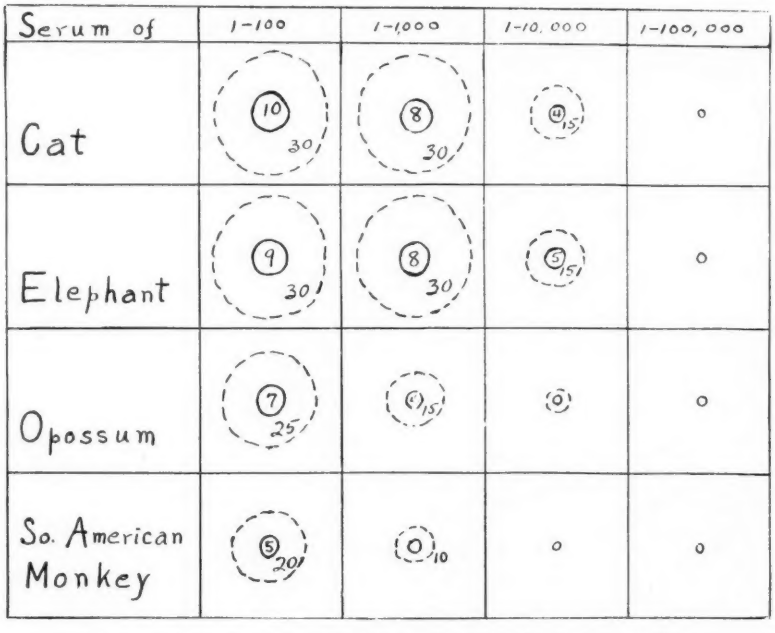

Fig. 1.—Scratch tests on H. D. with animal serums diluted.

Table I is an interpretation of the notes and is made simply to save space.

In addition to tests with animal serums, H. D. was tested with extracts of ground raw meat from various animals. Beef, pork, and mutton all gave typical responses to a crude extract in carbolized saline. Chicken, mackerel, codfish, and salmon, however, were entirely negative. In another experiment, beef muscle (steak) was cooked to various degrees before extraction and when "well done" failed to give positive tests.

An eye test made by dropping diluted horse serum into H. D.'s eye showed no reaction with the 1:10,000 dilution and only the slightest redness with the 1:1,000 dilution. A well-marked redness and itching followed the instillation of a drop of the 1:100 dilution.

In the meantime, it should be emphasized that H. D. did not react to "everything," for many substances were applied and with quite negative results.

Table I shows that the serum of every mammal, including the elephant and opossum, gives skin tests which are approximately uniform in

TABLE I

SCRATCH TESTS ON H. D. WITH ANIMAL SERUMS DILUTED

	1:100	1:1,000	1:10,000	1:100,000
Horse	+++	++	+	0
Guinea pig	+++	++	+	0
Cattle	+++	++	+	0
Sheep	+++	++	+	0
Hog	+++	++	+	0
Dog	+++	++	+	0
Rabbit	+++	++	+	0
Rat	+++	++	+	0
Mouse	+++	++	+	0
Cat	+++	++	+	0
Elephant	+++	++	+	0
Opossum	+++	++	+	0
South American monkey	++	+	0	0
Monkey (Macacus)	+	+	0	0
Man, Group II	0	0	0	0
III	0	0	0	0
IV	0	0	0	0
Frog	0	0	0	0

The symbols +++–0 refer to various sizes of wheal and erythema reactions. See text and see Fig. 1.

size. Monkey serum gives smaller reactions than the others. Human serum is negative as expected. Frog serum, however, is also negative.

The power of H. D.'s serum to transfer sensitiveness passively to local skin sites on a normal recipient was tested and found to be positive with each mammalian serum.

In the second experiment, a study of the power of different animal serums to desensitize the passively sensitized sites was made. Sites on the skin of a normal recipient were sensitized with H. D.'s serum and then in twenty-four hours were injected with one serum or another, always in 1:100 dilution. An immediate response was obtained which faded slowly. On the second day (forty-eight hours), the same diluted serum was injected into the same site again. The response was usually smaller. On the third day, a third dose was given, and usually there was no reaction at all, but occasionally four doses were required to produce complete desensitization. When finally this was accomplished, two days later some other serum also in 1:100 dilution was injected into the desensitized spot. The results are shown in Table II.

Table II indicates clearly that sites desensitized with guinea pig serum are desensitized to all other serums. Horse serum and cattle serum desensitize to each other. Horse dander will desensitize to horse serum, but sites desensitized with horse serum will still react to horse dander.

A refractory condition of the local skin areas following the repeated doses was considered but excluded by testing them later with horse dander and finding that they were capable of reacting to it.

These experiments indicate that normal skin sites treated with the serum of H. D. are sensitized to a substance which has a wide distribution among the common mammals but is not present in detectable concentration in the serum of man or in the serum of the nonmammals tested (chicken, frog, mackerel, codfish). The particular substance is present in horse dander as well as in horse serum. In horse dander, however, there is, in addition to this common substance, a special element with a specificity of different character.

TABLE II

DESENSITIZATION OF SKIN SITES PASSIVELY SENSITIZED WITH H. D.'S SERUM

DESENSITIZED WITH	TESTED WITH	RESULT OF TEST
1. Guinea pig serum	Horse serum	Negative
2. Guinea pig serum	Guinea pig serum	Negative
3. Guinea pig serum	Cattle serum	Negative
4. Guinea pig serum	Sheep serum	Negative
5. Guinea pig serum	Hog serum	Negative
6. Guinea pig serum	Dog serum	Negative
7. Guinea pig serum	Rabbit serum	Negative
8. Guinea pig serum	Rat serum	Negative
9. Guinea pig serum	Mouse serum	Negative
10. Horse serum	Cattle serum	Negative
11. Cattle serum	Horse serum	Negative
12. Horse serum	Horse dander	Positive
13. Horse dander	Horse serum	Negative

Third, a study of the chemical nature of the common serum antigen to which H. D. was sensitive was begun with experiments on horse serum and certain other substances. Heating horse serum to 56° C. for one hour did not change its skin-test-producing property, but placing it in boiling water for thirty minutes reduced this activity at least 100 times; that is, no positive skin test was obtained with dilutions higher than 1:10, whereas before heating, the test with the 1:1,000 dilution was definite. Heating beef serum gave similar results. Boiling of beef muscle and mutton muscle for thirty minutes in water destroyed the activity of the filtrates.

The antigen was precipitated by complete saturation with ammonium sulphate, but not by half saturation and is, therefore, not a globulin. It is present, however, in concentrated antipneumococcic globulin solution but in lower concentration than in normal horse serum. A dilution of antipneumonic serum, 1:100 gave a slight skin test, but the dilution of 1:1,000 was negative.

Since the Forssman antigen is soluble in strong alcohol, normal horse serum was treated as follows: It was dried and then extracted with various proportions of alcohol in distilled water. The extracts were

then evaporated to dryness and taken up in saline. This treatment with alcohol gave diminishing tests as more alcohol was used. The 50 per cent extract gave a positive test, but no result was obtained with extracts made with concentrations of 80 per cent and higher. Evidently, the antigen is not identical with the Forssman antigen. It is not a globulin. Further study of its chemical nature is needed.

Granted that an antigen common to the serum of many mammals exists, how did H. D. become sensitized to it? There are three possibilities: First, that he had received a previous injection of animal serum. Careful questioning brought only denials of the possibility. Second, that the inhalation of dander sensitized him to serum. The desensitization experiments described above indicate that horse dander and horse serum contain a common serum antigen and that in addition the dander has a specific antigen of its own. The dander element is not present in the serum. We must accept exposure to the dander as the origin of his hypersensitiveness to the dander element. Being sensitive to the dander, one would suppose that he would be sensitive *ipso facto* to the serum antigen and thus react to horse serum as well. The fact is, however, that all patients sensitive to horse dander are not sensitive to horse serum. Indeed, very few patients with horse asthma give a strongly positive skin test to horse serum. Evidently, they are sensitive to the dander element alone. H. D. like most patients with horse asthma shows skin tests to the dander not only of horses, but also of cats, rabbits, and dogs. If we contemplate a common antigen in the serum, must we contemplate another common antigen for the various danders? The subject becomes very complicated and demands much further study. The question naturally arises, is H. D. an exceptional case, or could others like him be found?

Scratch tests, with a series of ten different animal serums, all in 1:10 dilution, were made on eleven other patients sensitive to horse serum. Five were sensitive to horse serum alone. Four were sensitive to horse serum plus one or two additional serums. Only two were sensitive to all nine mammalian serums.

A third possibility is suggested by the positive skin tests to beef and sheep muscle; that sensitiveness to the common antigen arose through the absorption of this antigen by eating meat. The evidence that proteins can be absorbed unchanged through the intestinal wall has been reviewed recently in great detail by Ratner who, as a result of his study, believes that "under normal conditions, absorption of unaltered protein occurs with great regularity."

To explain these various findings, the following theory is suggested: The serum of mammals contains a common serum antigen which differs from the familiar species-specific antigen characteristic of each one. Most patients become sensitive only to this latter species-specific

factor, but a few individuals like H. D. become sensitive to the common factor and, therefore, show positive skin reactions to a wide variety of material derived from animal sources. A great deal of work remains to be done, and this paper is offered merely as a preliminary report. The observations discussed in it may be tabulated in the following summary:

SUMMARY

1. A patient with vasomotor rhinitis was found to be highly sensitive to horse serum and equally sensitive to the serums of a variety of different mammals.

2. Local passive transfer of hypersensitiveness to these serums was successful.

3. Skin tests to the raw muscle extract of chicken, mackerel, codfish, and to the serum of the frog and of men who belonged to different blood groups were negative.

4. The identity of the antigenic principle present in the various mammalian serums which gave positive skin tests was apparently demonstrated by desensitization of passively sensitized skin sites.

5. The antigen is not soluble in strong alcohol; it is precipitated by complete saturation with ammonium sulphate but not by one-half saturation. Boiling destroys it to a great extent but not completely. It is not affected by heating to 56° C. for one hour.

6. The presence of a species nonspecific antigenic factor in mammalian serums is not incompatible with the existence of other factors which are species specific.

7. The source of exposure responsible for this patient's hypersensitiveness to serum is discussed. The inhalation of horse (or other mammalian) dander and the ingestion of mammalian meat are considered.

8. Intradermal tests with serum in concentration greater than 1:1,000 may be dangerous.

9. The intradermal test in this case was about one thousand times more sensitive than the scratch test.

10. The conjunctival test was somewhat less sensitive than the scratch test.

11. A purified horse serum globulin solution contained the antigen but in much lower concentration than normal horse serum.

12. Antiserums made from mammals other than the horse will not necessarily eliminate serum accidents.

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DISCUSSION

DR. GRAFTON TYLER BROWN, Washington, D. C.—I rise to mention one interesting case that seems pertinent to the discussion. One of my asthmatic patients, a young girl, was found to be sensitive to beef, goat meat, horse meat, and other meats, and also to beef serum, goat serum, and horse serum. This is the only patient I have seen who reacted to so many different meats, as well as to the corresponding serums.

DR. FRANCIS M. RACKEMANN, Boston, Mass.—As a matter of fact, in spite of the program, I cannot take credit for this work, much as I should like to, for it was Dr. Simon's idea and it was all his work. It interests me, naturally enough, and to my mind the important part of it is, the absorption experiments that he described, how it is that passively sensitized sites, desensitized with one serum, will not react with another, and that fits in with what Dr. Tuft described and Dr. Unger described this morning. We will have more information about this subject from now on.

DR. BEN RAPPAPORT, Chicago.—Yet, in animal experimentation, I mean with precipitin work, there is a remarkable specificity of all of these serums. That is the thing most interesting to me and quite unexplainable. The only solution I can see is that apparently there is certainly a definite difference in this work in the specificity of precipitin reactions in animals and the Prausnitz-Küstner antibodies in man, as far as these serums are concerned.

DR. ARTHUR F. COCA, New York City.—Could the sensitivity to the monkey serum be transferred?

DR. SIMON.—The test was done with monkey serum, not monkey dander, and we tested this patient with all the other serums before we were able to secure some monkey serum, so that was put on at the last moment, and we did not use it in our desensitization experiments.

I think there are several things that should be done before this theory of a common antigen is accepted, although other common antigenic factors are well known. For instance, most of us accept the idea of the common antigenic factor in the grass pollens, and there are common antigenic factors in bacteria. The Forssman antigen is well known. I noticed in some work done in 1929 by Cooke and Spain, when they were studying serum hypersensitiveness that they were able, in a few cases, to transfer this hypersensitiveness passively to the skin of a normal person. In making the test in this prepared area they used horse serum and got a positive reaction. In order to prove the specificity of this transfer, they tested another prepared area with egg white and got a negative reaction. However, on one occasion, and they recorded this as a footnote down at the bottom of the page, they used rabbit serum as a control, and in this case, to their surprise, they found that there were reagins to rabbit serum as well as to horse serum, although rabbit serum was not able completely to desensitize to the horse serum, but horse serum did completely desensitize the site to rabbit serum. There must have been a common antigenic factor present.

My idea now is that instead of using desensitization of a passively sensitized skin site, it might be better to use the test tube neutralization

technic which Walzer described, because in this way one does not have to inject a skin site repeatedly to desensitize it.

There are one or two other points, I think. The specificity of an antigen, as Landsteiner and others have shown, does not depend upon its biologic origin but upon its chemical composition; and it is not unreasonable to suppose that there may be certain radicals in a protein molecule which are nonspecific, and others which are specific. Some people may become sensitized to the specific part, but perhaps a few may become sensitized to the nonspecific part of the molecule, which might be imagined as extending throughout a certain group of animals and not in other animals.

Since this patient had a general reaction from an intradermal injection of 0.1 c.c. of a 1:100 dilution of guinea pig serum, my idea is that in testing a patient for serum hypersensitiveness, a skin test done with horse serum, intradermally, should not be stronger than 1:100 and preferably only 1:1,000 dilution for the first test. If you want to give stronger serum later on, all right.

PRIMARY GRANULOCYTOPENIA DUE TO HYPERSENSITIVITY TO AMIDOPYRINE*

THEODORE L. SQUIER, M.D., AND FREDERICK W. MADISON, M.D.
MILWAUKEE, WIS.

ON OCTOBER 27, 1933, before the Central Society for Clinical Research, we reported[1] evidence to show that the taking of drugs containing amidopyrine (pyramidon) played an important rôle in the etiology of some cases of acute primary granulocytopenia (agranulocytic angina).

In each of 14 patients the onset was directly preceded by the use of amidopyrine alone or in combination with a barbiturate. The mortality in a group of six patients who continued the use of drugs containing amidopyrine was 100 per cent, while in a group of eight patients who did not continue the use of these drugs, only two died, and both of these died in the initial attack.

We have since observed[2] six additional cases, five of which definitely followed the use of amidopyrine, while the sixth followed the use of powders said to contain acetanilid, but the actual composition of which was undetermined.

Watkins[3] reviewed a series of 32 cases of granulocytopenia and found that 24 patients had taken amidopyrine or a barbiturate derivative for varying periods before the onset of the illness.

Grant[4] reported a patient with arthritis who had taken amidopyrine regularly for several months and discontinued the drug on admission to the hospital for cholecystectomy. During convalescence she was given a sedative containing amidopyrine and a few hours later had a chill followed by transient unexplained fever. Blood counts were not made. A few weeks later she again started taking amidopyrine and immediately had another chill and rapid rise of temperature. On the following morning her leucocyte count, which had been normal four days previously, was 2,000 with 2 per cent granulocytes.

Hench[5] reported a patient with chronic infectious arthritis who on the sixth day of medication with small doses of amidopyrine had a mild fever. Her white blood count which had been 8,600 was found to be 2,100. All drugs were stopped, and five days later the white blood count had returned to 6,000.

Randall[6] reported alarming leucopenia following the use of amidopyrine and phenobarbital. He attempted to evaluate the possible allergic factor in his patient by injecting small amounts of each drug,

*Read before the Twelfth Annual Meeting of The Association for the Study of Allergy, Cleveland, June 11, 1934.

both separately and combined, in saline solution. Local reactions were slight, and no appreciable change in the leucocyte count was noted.

Hoffman, Butt and Hickey[7] reported a series of 14 consecutive cases in which 13 patients had taken amidopyrine alone or in combination with codeine before the onset, and the fourteenth patient developed agranulocytic angina after two weeks' ingestion of dinitrophenol.

Zinninger[8] referring to two fatal cases previously reported by her, added that both patients had used an undetermined, but quite large quantity of amytal compound (amytal with amidopyrine), over fairly long periods of time.

Holten, Nielsen and Transbøl[9] reported six fatal cases all of which developed after amidopyrine medication in women during hospitalization for other disorders. They concluded that in all probability amidopyrine in therapeutic doses is, under circumstances not yet explained, capable of producing agranulocytosis.

The accumulated evidence leaves little room for doubt that amidopyrine in certain individuals is capable of producing the blood picture and clinical symptoms of acute primary granulocytopenia.

Drugs of the type represented by amidopyrine are recognized as frequently responsible for various manifestations of drug hypersensitivity, and we feel that the granulocytopenia observed after amidopyrine is the result of such a reaction. In support of this hypothesis we have produced granulocytopenia at will on readministration of amidopyrine to individuals who have had the acute disease following use of this drug. Furthermore, in two patients we have obtained positive skin reactions to patch tests with amidopyrine with depression of the granulocyte count and clinical symptoms identical with those observed on administration of the drug by mouth. We wish to report these two cases in detail.

CASE 1. A forty-year-old male physician gave a history of severe dermatitis of the legs in 1923 subsequent to the use of picric acid preparatory to operation for varicose veins. Otherwise his past history was essentially negative. On November 16, 1931, the characteristic symptoms of granulocytopenia developed, and his white count was found to be 2,100 with complete absence of granulocytes. After his recovery we found that immediately preceding the onset of the illness he had taken allonal (allyl-isopropyl-barbituric acid with amidopyrine) and that for some time previously had been in the habit of taking that drug frequently for restlessness and insomnia. On May 17 and July 5 there were two sharp recurrences with granulocyte counts of 500 and 390 respectively. The first recurrence was attributed at the time to the use of radium in his surgical work, and the second was unexplained. It was later found that amidopyrine had been taken immediately preceding these recurrences. After his

count had maintained a normal level for ten months, he was given a single 5 grain (0.3 gm.) tablet of amidopyrine. Within three hours he complained of a chilly sensation, deep-seated bone ache and malaise and his temperature rose to 102° F. In twelve hours the white count fell from the previous level of 7,800 to 2,000 and the granulocytes from 4,750 to 250. Four days later the white count was 6,750 with 2,340 granulocytes and after six days was 7,800 with 4,530 granulocytes.

There were no further recurrences, and on December 12, 1933, patch tests were made using a 10 per cent suspension of amidopyrine, amytal, amytal with amidopyrine, phenobarbital, aspirin, and allonal (allyl-isopropyl-barbituric acid with amidopyrine) applied in the usual manner to the unabraded skin.

Twenty-four hours after application of the patch tests he complained of tiredness and aching of the bones. The patches were immediately removed, but the symptoms increased, and four hours later he complained of chilly sensations, his temperature was 102° F. and the aching severe. The white count immediately after removing the patches was 5,450; four hours later it was 3,550, and the following morning, thirty-six hours after the patches were applied, it was 2,700. During the next forty-eight hours the count gradually returned to the normal level of 7,200. The amidopyrine and allonal patches both showed definite reactions, and twenty-four hours later a delayed reaction was present at the site of the amytal with amidopyrine and the phenobarbital patches. All others were negative. Prausnitz-Küstner tests were negative.

Case 2. A thirty-four-year-old housewife gave a history of having been in bed for eighteen months with chronic infectious arthritis which had been progressive since its onset in July, 1931. On admission to Milwaukee County Hospital August 25, 1933, there was polyarthritis with considerable deformity and mild elevation of blood pressure. Her white count was 5,550 with 4,330 granulocytes. She was given amytal compound (amytal with amidopyrine) and sodium salicylate on August 25 and 26, amytal compound and cinchophen on August 27 and 28, cinchophen alone from August 29 to September 6, and amytal compound alone from September 6 to 12 inclusive. On September 9 her white count was found to be 1,800 with 340 granulocytes. On September 11 it was 1,300, and on September 12, 1,100 with 110 granulocytes. At this time ulcers appeared on the tonsils and her temperature was 99.4° F. Amytal compound was stopped, and she was given nucleotide. On September 13 her white count was 1,050 with 60 granulocytes. The following day her total count rose to 4,900, and improvement continued until on September 18 it was 12,500 with 8,750 granulocytes and it remained between 7,000 and 10,000 subsequently. On October 10 she consented to a trial administration of

amytal compound. Blood counts made at 10 A.M. and 4 P.M. on this day showed 8,200 and 8,450 white blood cells with 5,160 and 6,850 granulocytes, respectively. A single 10 grain capsule of amytal compound (amytal 3 grains, amidopyrine 7 grains) was given by mouth. The following morning she complained of being chilly and generally uncomfortable, and her temperature which previously had been normal rose to 99° F. at 9 A.M. The white blood count at this time was 2,750, and at 2 P.M. was 2,250 with 1,350 granulocytes. The following day her total and granulocyte counts rose to 3,700 and 2,180 and counts on the thirteenth and fourteenth were 6,950 and 11,100 respectively with normal granulocyte levels.

On October 24, after another interval during which the white count remained normal, she was given 3 grains of amytal alone. No significant alteration in either the total or granulocyte count was observed.

On November 27 she was given 5 grains of amidopyrine by mouth. Before administration of the amidopyrine her white count was 10,000 with 7,400 granulocytes. Twelve hours later her count fell to 6,050 and after sixteen hours to 3,850. Again she complained of chilly sensations, and her temperature which previously had maintained a normal level rose to 99° F.

On January 5, 1934, her total white cell count was 12,000 with 8,800 granulocytes. The same series of patch tests was applied as in Case 1. Twenty-four hours later her count fell to 7,500 and in forty-eight hours to 5,900, and the granulocytes to 5,850 and 3,950 respectively. At this time the patches were removed, and mild but very definite reactions were present at the sites of application of amytal compound, amidopyrine and phenobarbital. Twenty-four hours later a delayed reaction was present at the site of the allonal patch. Counts made two hours and five hours after removal of the patches both showed 5,900 white cells with corresponding granulocyte counts of 3,680 and 3,420. The following day, twenty-one hours after removal of the patches, the total count had risen to 9,500 and the granulocyte count to 5,890. Her temperature, which had been constantly normal for days before testing, rose to 99.8° F. when her count fell to 5,900 but no definite subjective symptoms were noted.

Although most of the reported cases of acute primary granulocytopenia following the use of drugs have been in relation to those containing amidopyrine, some have been reported after other drugs, such as arsphenamine and gold salts.[10-14]

An especially significant case has been reported by Stratton[15] in which granulocytopenia followed arsenobenzene therapy, and after recovery arsenic medication was resumed using a pentavalent instead of a trivalent compound without further reaction.

In the two specific cases here reported as well as in most of the

larger series mentioned, contact with amidopyrine prior to the illness has been established, and on readministration of amidopyrine we have been able to produce the characteristic disease picture at will. Patch testing not only yielded positive skin reactions but was accompanied by a characteristic systemic reaction. Furthermore the amount of amidopyrine that could possibly be absorbed from a few drops of a 10 per cent suspension applied to the unabraded skin must be so small as to preclude any possibility of toxic or pharmacologic drug action.

Kracke and Parker[16] reported a series of 11 cases, 6 of which followed the use of amidopyrine, and have formulated the hypothesis "that the depressant effect on the granulocyte count is produced, not by the direct action of benzene upon the bone marrow, but by the action of some of its oxidation products."

Because in animals injected with benzene derivatives, neutropenia was produced in only an occasional animal, they postulate inferior bone marrow in the animals affected and believe that in human beings an inferior, weakened bone marrow must be presupposed in the production of granulocytopenia.

It is well known that leucopenia is frequently present in allergic reactions to drugs just as in serum disease, and a chill, elevation in temperature, and muscular pains are also common.

We believe that the blood changes in acute primary granulocytopenia are best explained as the result of the repeated administration of certain drugs, especially amidopyrine, to persons who have developed specific allergic drug hypersensitivity. The aching, exhaustion and fever common to many drug hypersensitivities, and observed in our cases, are symptoms that would frequently lead to readministration of such an analgesic as amidopyrine. It is our conception therefore that certain individuals who frequently resort to amidopyrine or its various compounds develop hypersensitivity to that drug. The allergic symptoms produced by subsequent ingestion are such that the drug is repeated with resulting continued and increasing depression of the granulocytes. Finally, profound leucopenia makes possible bacterial invasion of tissues and the development of lesions of the mucous membranes to complete the clinical picture of acute primary granulocytopenia.

SUMMARY

1. In a series of 19 patients observed by us the onset of acute primary granulocytopenia was directly preceded by the use of amidopyrine alone or in combination with a barbiturate. A similar relationship has been reported in the literature and by personal communication in 52 additional cases.

2. The administration of a single dose of amidopyrine to each of two patients who had recovered from the acute disease was followed by a rapid profound fall in the total and granulocyte counts.

3. All patients under our observation who recovered from the acute disease and avoided the use of amidopyrine have had no recurrences over periods of from twelve to thirty-one months.

4. Skin sensitivity to amidopyrine was demonstrated in two of these patients by patch testing, and systemic reactions with marked depression of the total and granulocyte counts and elevation of temperature were present in both after application of a 10 per cent solution of amidopyrine to the unabraded skin.

5. On the basis of this evidence we believe that acute primary granulocytopenia can be caused by repeated administration of amidopyrine to persons who have developed allergic or anaphylactoid hypersensitivity to that drug. From analogy we believe that certain other drugs may be responsible for a similar granulocytic response.

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DISCUSSION

DR. HARRY L. ALEXANDER, St. Louis.—May I ask whether in the history of these patients it is evident that all of them had taken this drug or its derivative at some previous time? Is it possible that symptoms occur after continuous administration without previous ingestion of the drug involved?

DR. HORACE S. BALDWIN, New York City.—I should like to ask Dr. Squier whether any other patients he had were sensitive to the drug mentioned and failed to give positive patch tests and whether he found any patients who had a leucopenia following amytal or phenobarbital.

DR. SAMUEL M. FEINBERG, Chicago.—I should like to ask Dr. Squier whether he has an explanation for the fact that he got positive patch tests and negative skin tests in this particular group of cases.

DR. MARION B. SULZBERGER, New York City.—My question is going to exhibit my ignorance. I should like to ask the doctors why they consider pyramidon a benzene derivative. I thought that amidopyrine contained not a benzene, but a pyrazolon ring. Is there any absolute proof that this splits into a benzene ring in the body?

I, for one, have never doubted that blood dyscrasias can be due to drugs. In the cases of blood reactions due to arsenobenzol, I have repeatedly expressed this opinion.

My friend, Dr. J. Wolf of New York, recently called my attention to the fact that drugs which cause granulocytopenias are just those which cause fixed drug eruptions, e.g., the arsenobenzols, the gold compounds, phenacetein, amidopyrine; these are the main offenders in both conditions. In this connection we thought it would be interesting to investigate the action of phenolphthalein on the blood picture. All of us who have observed such reactions to drugs cannot doubt that they are due to idiosyncrasy and sensitization. It seems quite possible that fixed, circumscribed, and localized areas in the bone marrow may be the shock tissue. However, there are other explanations possible.

I should like to express no verdict on the results of patch tests which the authors report. The findings are without precedent, most unusual, and somewhat contradictory to the accepted concepts. I believe it would be well to be cautious in drawing conclusions from these as yet isolated observations.

This work of Dr. Kracke and Parker and of Dr. Squier and Dr. Madison is, I think, a very important and interesting contribution. I wish it had been made several years ago. If that had been the case, my old chief, Professor Bruno Bloch, might be alive today. He constantly took preparations containing amidopyrine, and he died of agranulocytic angina.

DR. E. WOOD PHILLIPS, Phoenix, Ariz.—I should like to mention that in the past winter I saw three cases of agranulocytic angina, so-called, all in persons who were proved to be allergic.

The first was a man who was both food and pollen sensitive and who, for arthritis, had been taking allonal at various times for some six weeks. The second was a nurse who had had a streptococcus infection causing arthritic symptoms and took "Peralga" off and on

whenever her joints hurt. She was known to be sensitive to certain foods. This patient died.

The third was a particularly interesting case, an overweight young woman who had been very healthy except for headaches. She was food sensitive. She had at times taken some headache medicines; but never before an accident that she had, had she taken any allonal. While taking some reducing exercises she dislocated her shoulder and was given allonal for relief of pain. In a period of five weeks she took 21 tablets. She remained for nine days without any granulocytes and finally recovered after the eleventh transfusion.

DR. BEN RAPPAPORT, Chicago.—Will Dr. Squier speak of the danger of such experimental work? He seemed to be quite confident in carrying on the experiment in these two cases that the leucopenia produced would be temporary.

DR. SQUIER.—Dr. Alexander asked whether granulocytopenia has occurred during continuous administration of amidopyrine. Our second patient developed granulocytopenia while taking amidopyrine, and then after recovery we demonstrated a drop in the granulocyte count upon readministration of the drug. In our animal experiments previously reported one rabbit developed granulocytopenia after long administration of allonal; and, interestingly enough, two separate depressions of the total and granulocyte levels were observed. Both followed readministration of the drug after omission for a day.

Dr. Feinberg asked about intracutaneous testing. We have not done it on our patients because we have not dared to do so.

In answer to the question of Dr. Sulzberger relative to the benzene ring, amidopyrine is a pyrazolon derivative of benzene and contains the "benzamine" group, and oxidizes finally to quinone, as has been pointed out by Kracke.

I should like to say to Dr. Rappaport that we have approached testing in all these patients with fear and trembling. We were astounded at the violent reaction after patch testing in the one case reported. We avoided intracutaneous testing because we felt it was dangerous. On the other hand, we feel it is perfectly safe to test first by the patch method and, if there is no appreciable depression of granulocytes, to test further by the administration of a single tablet by mouth. In all of the instances observed by us, although depression of the count was severe, there was return to normal within the course of forty-eight hours. There is relatively little danger in the administration of a single dose unless an active infection is present. In such a case under no circumstances would we attempt testing.

We have not investigated the allergic background of all of our group. The patient who had chronic arthritis (Case 2) did not have an allergic background. The physician (Case 1) had had vasomotor rhinitis for years, but skin tests were negative. It is interesting that during the height of his reaction, following patch testing his nose became almost completely occluded so that breathing was difficult, and that there was immediate relief after administration of 0.5 c.c. adrenalin.

A REVIEW OF THE PHASES OF ALLERGY

FRANCIS M. RACKEMANN, M.D.* †
BOSTON, MASS.

THE present interest and increasing knowledge of allergy and its treatment have depended very largely upon a study of the trigger mechanism which excites a variety of reactions in different subjects. The particular kind of reaction which results depends in part upon the chemical nature of the exciting substance—the allergen—but it depends also upon conditions in the host.

At the meeting of the Association for the Study of Allergy in 1933, Dr. Caulfeild[1] reviewed our knowledge of the chemistry of those antigens which give symptoms in allergic individuals and which produce positive skin reactions. This year it seems appropriate to attempt a review of the host factors.

Allergy is at least one striking condition in which a study of the individual who suffers from the disease is more important than a study of the agent which causes that disease. In fact, allergy as a whole depends upon changes in the host, which we speak of as sensitization and which are determined by the nature of the previous contact with the specific substance. To say that a reaction is allergic is to imply that the individual who manifests that reaction has had previous contact with the specific substance.

There are three types of reaction which are spoken of as allergic, and the relation between them is of greatest importance. Some of the recent studies on the subject are summarized in Table I.

The studies of Dienes[2] are most valuable. When the normal guinea pig is injected with serum or egg white intraperitoneally, intravenously, subcutaneously or intracutaneously, according to the tenets of experimental anaphylaxis and then is tested from day to day by repeated intracutaneous injections of the same substance, the first few tests if carefully studied will be seen to produce a red, inflammatory reaction which first appears in twenty-four hours after the skin test. Later, however, when several daily skin test doses have been given, a reaction of the immediate type accompanied by diffuse swelling will appear, and at that same time precipitins will be found to have developed in the animal's blood. In case, however, this same experiment is repeated on a tuberculous guinea pig and particularly if the doses of egg white are injected into the tuberculous lesion itself, usually the

*From the Anaphylaxis Clinic of the Massachusetts General Hospital.
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lymph nodes, then the successive intradermal doses of egg white will show another picture. The second or third dose given within a few days will reproduce an active response with redness, swelling, and inflammation soon going on to necrosis. If the doses of egg white be continued, the later response will be earlier in time and less inflammatory in nature. As Dienes explains, the first phase with inflammation and necrosis represents the tuberculin type of reaction produced now by egg white, whereas the later phase represents the immediate or

TABLE I

REVIEW OF ALLERGIC REACTIONS

	METHOD OF SENSITIZATION	TYPES OF REACTIONS OBSERVED				
		TUBERCULIN		ANAPHYLACTIC		ECZEMATOID
		S. T.	A.	S. T.	A.	P. T.
Experimental	*Protein Anaphylaxis* (g. pigs)	+	0	+++	++	0
	In infect. lesion (Dienes)	+++	0	+++	+++	
	Intradermal serum, egg	+	0	+++	++	0
	(G. pigs) ragweed	+		++	+	0
	neoarsphenamine	++	0	0	?	
	Intradermal (man – G. P. S.)	+	0	++	P. K.	
	Contact Dermatitis (man)	0	0	0	0	+
	Rub g. pigs Ursol	0	0	0	0	+
	Paint g. pigs poison ivy	0	0	0	0	+
Clinical	*"Natural" allergy* H. F., A., E.	+	0	+++	P. K.	0
	Drug allergy	S	0	S	S	S
Infection	*Serum disease*	+	0	+++	++	
	Acute infection (pneumonia)	+	0	++	++	
	Chronic infection (T. B.)	+++	0	+	0	+
	"-Ids" (Trichophyton)	++	0	?	0	+

+ = Positive. O = Negative. S = Sometimes. ? = Unknown.
S. T. = Skin Test. A = Antibodies. P. T. = Patch Test.
P. K. = Prausnitz-Küstner.
G. P. S. = Guinea pig serum.
H. F., A., E. = Hay fever, asthma, eczema.

anaphylactic reaction as seen in the normal animal. Antibodies like precipitins and passive transferring bodies do not occur in the early stage but appear only when the reaction becomes immediate and edematous. Evidently, antibodies play a part in this late phase.

Why the production of an inflammatory lesion should be so important for the development of the tuberculin type of sensitiveness is not clear, but it fits in with what we know about the tuberculin reaction and other bacterial tests. As Dienes says (1928 and 1932), it is probable that the pathologic tissue gives rise to antibodies of a certain kind. In an earlier discussion of this subject, I[3] referred to the inflammatory focus as giving rise to a "third factor" which influences the behavior of the animal, and now Dienes has a similar conception, but his name "the preparation factor" is much better.

The eczematoid reaction belongs in a special group. It can be discussed to better advantage later. In protein anaphylaxis it is negative.

During this past year, Dr. F. A. Simon[4] has been working in our laboratory on this same general problem, studying especially the intradermal method of sensitization. Previous results are confirmed. Repeated doses of horse serum or egg white given to guinea pigs intradermally result at first in a red inflammatory tuberculin-like reaction and later in an anaphylactic reaction at a time when circulating antibodies appear.

Simon has studied ragweed in its ability to sensitize guinea pigs and has shown that whereas the production of sensitiveness to ragweed when attempted by intravenous or intraperitoneal methods is very difficult (Julia T. Parker[5] was forced to use enormous doses) if guinea pigs are treated by repeated doses given intradermally, then the same positive reactions and the same sequence of events are observed as with serum. From these experiments it appears that sensitiveness of the skin is produced only by treatment of the skin itself. Antibodies are found but only in the form of passive transferring bodies. Precipitins are not present, but the blood of the treated animal is capable of transferring sensitiveness to a normal guinea pig. Repeated attempts to demonstrate an eczematoid reaction following intradermal treatment have so far failed entirely. Painting, rubbing, and applying patches wet with the specific substance are always negative.

Neoarsphenamine presents a special case, but the principles are evidently nearly the same. This year Sulzberger and Simon[6] have shown that by repeated intradermal treatment, strong reactions of inflammatory type result from a relatively few doses. As their report shows, many of these reactions go on to necrosis. In spite of the severity of this early phase in sensitiveness, anaphylactic reactions were not observed, and so far, all their attempts to demonstrate antibodies have been negative.

When man is used in place of guinea pigs, the results are entirely comparable as shown by Jones and Mote[7] and by Simon and Rackemann.[8] The development of positive skin reactions first of the tuberculin type and later of the anaphylactic type following repeated doses of guinea pig serum given intradermally seems to be the rule; and when the anaphylactic reaction is well developed, the serum of the patient is capable of transferring the reaction to the skin of a normal recipient. The man behaves quite like the guinea pig. So far, other animals have not been studied except to show in a few instances that cats and rabbits can be made to develop an inflammatory reaction by repeated intradermal injection of egg white and of horse serum.

The group of contact dermatitis is closely related. Our patients who develop a seasonal dermatitis because of their skin sensitiveness to pollen oil or have an occupational dermatitis due to certain substances

like hexamethylenetetramine in the rubber industry (Burrage[9]), or to the heavy metals such as nickel or even gold, or have skin lesions produced by drugs as in the cases of pharmacists and physicians who are sensitive to such substances as formalin, novocaine, and a variety of dyes; these cases present an interesting group. Their reactions are allergic because they are not produced in normal persons and because they do not occur in the patients until a very definite period of exposure has elapsed. In all of these, scratch tests with the specific substance are always negative. No reactions of the tuberculin type or of the anaphylactic type are produced. The patch test, however, is always positive.

Recently, this same condition has been produced in guinea pigs. The experiments of Bloch[10] who sensitized guinea pigs to paraphenylendiamine have been confirmed. When guinea pigs are rubbed with a paste containing 2 per cent of this substance, the animals are changed so that later the simple painting of their skin with the same paste results in a violent inflammatory reaction of the eczematous type. Skin tests by the scratch method, however, are always negative. Recently, Simon and Rackemann[11] have been able to sensitize guinea pigs to poison ivy simply by painting the extract on the skin. When later the poison ivy extract was again applied, a definite eczematous reaction quickly followed. All attempts to produce this reaction by subcutaneous, intraperitoneal, or intravenous doses failed, and all attempts to demonstrate antibodies by passive transfer from one animal to another likewise failed.

The "natural" allergies are in some contrast to the conditions described above which are experimental and artificial. Immediate wheal and erythema, urticarial reactions of the anaphylactic type are the rule in hay fever, asthma and eczema. The symptoms depend upon lesions which are evidently quite like the skin test. There is edema of the nasal and conjunctival mucosa and perhaps of the bronchi; there is spasm of the smooth bronchial muscles; there is a fall in temperature and blood pressure, and a fall in total leucocytes. This reaction depends upon antibodies, for the serum of the patient when injected in the skin of a normal recipient will transfer a local sensitiveness to that recipient. Whether in its development the process of natural sensitiveness like that of artificial sensitization passes through an early stage of inflammatory-tuberculin-like reaction is hard to say. Occasionally such reactions are observed in the clinic—especially in ward patients studied on the next day. Vaughan[12] has referred to the clinical value of watching skin tests for twenty-four hours to look for delayed reactions. The subject needs further study.

Drug allergy belongs in this clinical category as well as in the artificial group already described. The subject is complex for several reasons. Many drugs are toxic, while others produce their harmful

effects only after the patient has had repeated doses of them. The similarities and differences between anaphylactic and anaphylactoid reactions have been carefully reviewed by Hanzlik[13] and need not detain us here; suffice it to say that in many cases of drug sensitiveness a positive patch test can be obtained, but that in many others no reaction of any kind will follow any of the various forms of skin test. In rare instances, however, reactions of the tuberculin type or reactions of the anaphylactic type may be demonstrated. Quinine, for example, may according to Stitt,[14] produce an immediate urticarial reaction in patients with malaria who have developed a sensitiveness to it. Therefore the designation of "S" meaning "sometimes" appears in Table I.

Suggestion can do no great harm if there is a little reason behind it. A third section marked "Infection" has been added to the table. The incubation period, the fever, the limited course, and the demonstrable antibodies make serum disease easily comparable to acute infectious processes; and there is a little evidence which points to them all as reactions of the anaphylactic type. In serum disease the increased capillary permeability with edema into the skin and effusion into the joints depends upon the development of antibodies. The serum of the patient can be precipitated by small quantities of the antigen (horse serum) and is capable of transferring the sensitiveness passively to the guinea pig. The development of an immediate skin test occurs at the same time with these circulating antibodies and persists for a long period—months or years after the antibodies have disappeared.

Similarly, after pneumonia, Sutliff[15] and others have shown that the development of specific precipitins occurs at the same time when the immediate skin test can be obtained with the specific carbohydrate fraction of the pneumococcus. The Casoni reaction in echinococcus disease is an immediate response which follows the intradermal injection of a small amount of echinococcus fluid in a patient who has or has had echinococcus disease. Ascaris confers a similar type of sensitiveness. The rashes of measles, scarlet fever, syphilis, and smallpox are quite like serum disease; but since each of them differs from the other, it seems inevitable that each of them must depend upon the particular effect of its own specific toxin and not upon a general or nonspecific reaction of the body as a whole. Nevertheless, the nature of these exanthemata is still under discussion, and a comparison of them with serum disease is always interesting.

The delayed inflammatory reaction of the tuberculin type is equally interesting. This reaction consists of a red swollen inflammatory area which appears not in a few minutes but is delayed for twenty-four or forty-eight hours. The original dictum of Krause[16] was "no tuberculin reaction without a tubercle." And so we find that the focus of active disease is necessary if the animal is to show a marked delayed reaction later.

Besides tuberculin in tuberculosis, delayed reactions have been obtained by Gay[17] with his typhoidin in patients recovered from typhoid fever, lutein in syphilis, mallein in glanders and similar products in other diseases—the Frei reaction in lymphogranuloma inguinale.[18] More recently, studies of delayed reactions to streptococci and their products have aroused wide interest. If we assume that the lesions in deeper organs are comparable to the results of the skin test, it is easy to see how the inflammatory changes in joints in arthritis could easily be regarded as of allergic origin; and then when Swift[19] calls attention to the great variety of organisms which are claimed to cause rheumatism, the conception of allergy offers a convenient way of explaining these diverse results by pointing to a mechanism applicable to them all.

Certain skin diseases may likewise fit into this category. C. M. Williams[20] was the first to recognize that certain lesions on the skin of patients with parasitic dermatoses were different from other lesions because cultures always failed to show a parasite in them, and he conceived the idea that these other lesions were allergic reactions to material absorbed from the original focus. They were "-ids" and the comparison between "-ids" (dermatids) and "-oses" (dermatoses) became interesting. What other conditions depend upon this late form of allergic reaction is not now apparent, but it is easy to believe that in the future the list of possibilities will be extended.

The poison ivy reaction is still different. Here the specific sensitiveness is demonstrated by a patch test, whereas the injection of the allergen into the skin or below it fails to elicit any response. The patch test must be left on the skin for at least twenty-four or, better, forty-eight hours but in that time a positive reaction will consist of a reproduction of the original skin disease.

As Dienes points out, it appears that the cutaneous epithelium plays a very definite and characteristic part in allergy. Incidentally, too, it is of considerable theoretical importance to know that sensitiveness of the skin may be limited sharply to this particular tissue and does not spread through the body as a whole. This point, however, needs more attention. Clinically, the patch test is of great practical importance because through it the cause of trouble in many patients with contact dermatitis has been recognized.

The relation between the patch test and the other skin reactions is hard to define. In most cases, they are independent of each other and appear to rest upon separate mechanisms. In tuberculosis, however, is the Morro reaction—positive with the application of a bacterial paste to the unbroken skin and in trichophytosis Sulzberger finds that a delayed tuberculin-like reaction following the intradermal injection of the antigen and the patch test with trichophytin may be positive together at the same time.

The contemplation of these several reactions and their relationship one to another leads to the tempting suggestion that the differences between chronic infections, acute infections, and allergy are perhaps referable to the behavior of the host in the presence of the foreign substance causing the disease.

Tuberculosis and arthritis with emphasis on a chronic granulomatous process with lymphocytic infiltration and skin reactions of the tuberculin type represent the early phase of the immune process, and the patient "sticks" in that phase. Pneumonia, scarlet fever, and acute infections generally with a varying pathology, represent the normal response of the host, who passes through a succession of phases and ultimately escapes into the end phase of complete immunity. In these infections, the anaphylactic phase with immediate skin reactions to the bacterial carbohydrate has drawn most attention. According to this theory, our patients with asthma do not improve because they "stick" in the late or anaphylactic phase. The immune process is not completed.

Such an idea sounds attractive, but it may be that its conception depends merely upon the emphasis which has been given to different reactions in the literature. In tuberculosis, for example, it may be that the carbohydrate residue of Zinsser will give an immediate urticarial skin reaction. In pneumonia, our chief interest is in the carbohydrate, but Sutliff has already shown that the pneumococcus protein will give a delayed tuberculin-like reaction in the early part of the disease, and therefore, consistent with the theory.

The whole subject is fascinating and contains many important problems. In its study, the vital element must be the technic.

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263 Beacon Street

FURTHER STUDIES IN SERUM ALLERGY

VI. Antigenic Relationship Between Horse Dander and Horse Serum Sensitivity*

Louis Tuft, M.D.
Philadelphia, Pa.

THE relationship between horse dander and horse serum sensitivity has for some time been a debated subject. The results of previous experiments upon this question have indicated either that the two substances were completely separate antigenically or that there was present in horse dander a small amount of the horse serum antigen. Thus in 1918 Rackemann[1] showed that a guinea pig sensitized to horse dander died in two minutes when reinjected with the horse dander extract, whereas a guinea pig sensitized to horse dander showed no symptoms upon being reinjected with horse serum. Similarly the guinea pig sensitized to horse serum when reinjected with horse serum died in two minutes; but the other animal sensitized to horse serum when reinjected with horse dander showed no symptoms.

In 1925 and 1926 Longcope, O'Brien and Perlzweig[2] sensitized a large series of animals and tested the uterine muscle for sensitiveness by the Dale method. Despite the delicacy of the technic, the animals sensitized with horse dander showed no reaction to horse serum, and the pigs sensitized with horse serum showed no reaction to horse dander.

In 1928 Forster[3] in a series of cross precipitation, uterine strip, and anaphylactic reactions came to the conclusion that there was an antigenic element common to horse dander and to horse serum, present in relatively small proportion in horse dander. This conclusion was based on the following findings:

(1) In cross precipitation tests involving an antiserum against horse serum and horse dander, much lower titers were obtained when horse dander extract was tested with the heterologous antiserum (horse serum) than when horse serum was tested with the horse dander antiserum.

(2) The uterine muscles of 2 of the 7 guinea pigs sensitized with horse dander extracts were only partially desensitized by horse serum so that a subsequent reaction could be obtained after the addition of horse dander extract.

*From the Clinic of Allergy and Applied Immunology, Department of Medicine, Temple University School of Medicine.

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(3) Of 12 guinea pigs sensitized to horse dander extract 10 developed typical anaphylactic shock upon reinjection of the extract; whereas of 15 guinea pigs sensitized to horse serum none developed typical anaphylactic shock upon reinjection with the horse dander extract. In some of the latter, however, evidence of partial desensitization seems to have occurred. Forster mentions the possibility that the horse serum may be present in dander as a contaminent—as a result of currying—but states that if the serum proteins were derived from such a source, different lots of dander would contain different proportions of them. Therefore three different lots were used and harmonious results were obtained from all three.

Ratner and Gruehl[4] in 1929 in similar experiments came to the same conclusions. These authors showed that in animals parenterally sensitized to horse serum it was difficult to produce anaphylaxis by reinjection with horse dander. On the other hand, in animals sensitized to horse dander, reinjection with horse serum produced a greater percentage of anaphylactic reactions; this was not, however, a regular phenomenon. When the sensitization was produced by the inhalation of horse dander dust, the proportion of crossed reactions was considerably reduced. From these experiments the authors suggest that the inhalation of horse dander may play a part in the problem of human hypersensitiveness to horse serum.

From a clinical standpoint a greater unanimity of opinion exists concerning the antigenic relationship between these two substances. By most writers it is usually stated that those individuals who are naturally or atopically sensitive to horse dander, constituting the so-called "horse-asthmatic" individuals, are almost invariably sensitive to horse serum. To quote Coca, Walzer and Thommen,[5] "The writers are not aware of any authentic instances of atopic sensitivity to horse serum which, at the same time, did not show some cutaneous reactivity to horse dander as well. The reverse relationship holds true less frequently."

In most of these references no mention is made of the nature or cause of these reactions. The exact definition of the terms naturally or atopically sensitive to horse dander and horse serum is therefore not clear. Thus it may refer either to reactions occurring in the horse-asthmatic individuals or to marked skin reactions occurring in atopic individuals. As previously mentioned, Ratner believes that the inhalation of horse dander produces in the atopic individual a reaction not only to horse dander but likewise to horse serum.

In reviewing these facts in relation to other data in connection with serum allergy, it seemed to me in the first place that the results of the various experiments could best be explained in the light of the experiments of Forster: namely, that the horse dander extract contained small amounts of horse serum protein; therefore when horse dander

was the sensitizing agent, enough horse serum was present to sensitize the animal so that reinjection of horse serum produced anaphylactic shock; whereas when the animal was sensitized to horse serum, not enough of this antigen was present in the dander extract used for reinjection to produce anaphylaxis. This was likewise true of respiratory sensitization with the horse dander dust.

Furthermore in reviewing the data relating to clinical sensitivity, it seemed also that insufficient attention had been paid to the rôle of previous injection or contact with either horse dander or horse serum or both, in explaining the existing sensitivity.

To provide, therefore, more definite data in relation to the problem of serum allergy in the human being, it seemed worth while to study this problem further. Two plans of investigation were adopted:

(1) Cross-neutralization experiments with human serum containing reagins to horse serum and horse dander, the serum being obtained from allergic individuals showing positive skin reactions to both allergens.

(2) The determination in such patients of possible etiologic factors to account for the sensitivity either to horse serum or horse dander or to both.

EXPERIMENTAL

Material.—For the first portion of this investigation, the serums of 8 asthmatic patients, showing marked skin reactions to both horse serum and horse epithelium, were employed. Horse serum antigen in dilutions of 1:10 to 1:1,000,000 and horse epithelium extract in concentrations of 0.001 to 0.5 total nitrogen, prepared according to the method of Coca, were used in these experiments.

The technic was that usually employed in cross-neutralization experiments by passive transfer: 0.1 c.c. of the serum was injected intradermally into the arms of 2 or 3 nonallergic individuals. After a twenty-four- to forty-eight-hour interval, one site on each person was injected with horse serum and a second with horse dander. After a similar interval, these were retested with the same allergens; if negative, the sites were reinjected after a similar interval with the opposite allergen. Proper controls with the two allergens were used in all instances, and only when these were negative were the results utilized. Quantitative determination of the antibody concentration was made in four of the serums. This was accomplished by testing the sensitized sites with varying dilutions of each allergen. In three of the patients results were checked by the test tube neutralization method of Walzer and Bowman.[6]

Results.—These are shown in Table I. From the latter, it will be seen that of the eight serums, exhaustion of the reagins to horse serum following desensitization of the sensitized sites by horse epithelium

TABLE I

PATIENT	AGE	PAST HISTORY: SERUM ADMINISTRATION	PAST HISTORY: HORSE CONTACT	ALLERGIC FAMILY HISTORY	SKIN TESTS: HORSE SERUM 1:100	SKIN TESTS: HORSE EP. 0.001 MG. N PER C.C.	EYE TESTS: HORSE SERUM	EYE TESTS: HORSE EP.	PASSIVE TRANSFERENCE SERUM SITES	INTERVAL IN DAYS: 1	2	3	4	5	6	QUANTITATIVE STUDIES: HORSE SERUM 1:100	H. S. 1:1,000	H. S. 1:10,000	HORSE EP. 0.001 MG. N PER C.C.	H. E. 0.0001 MG. N PER C.C.	H. E. 0.00001 MG. N PER C.C.
(1) J. W.	29	Negative			Marked	Marked	Marked		R.	H. E. 0.001 Marked	→ Mod.	H. S. 1:100 Negative	Negative								
									L.	H. S. 1:100 Marked	→ Mod.	H. E. 0.001 Marked	→ Marked								
(2) C. M.		1 course Toxin-Antitoxin		Positive	Marked	Marked	Marked		R.	H. E. 0.001 Marked	→ Slight	H. S. 1:100 Negative			H. S. 1:100 Negative						
									L.	H. S. 1:100 Marked	→ Slight	H. E. 0.001 Marked			H. E. 0.001 Marked						
(3) R. F.	12	Diphtheria Antitoxin 1927 & 1929	11 yr.		Marked	Marked	Marked	Marked	R.	H. E. 0.001 Marked	→ Negative	H. S. 1:100 Negative				Marked (test-tube neutralization gives same results)	Slight	±	Marked		
									L.	H. S. 1:100 Marked	→ Negative	H. E. 0.01 Marked									
(4) G. K.	39	Negative	Entire life		Moderate	Marked			R.	H. E. 0.01 Marked	→ Negative	H. S. 1:100 Negative				Moderate	Moderate	Slight	Marked	Moderate plus	Slight plus
									L.	H. S. 1:100 Marked	→ Negative	H. E. 0.01 Marked									

TABLE I—CONT'D

PATIENT	AGE	PAST HISTORY: SERUM ADMINISTRATION	PAST HISTORY: HORSE CONTACT	ALLERGIC FAMILY HISTORY	SKIN TESTS: HORSE SERUM 1:100	SKIN TESTS: HORSE EP. 0.001 MG. N PER C.C.	EYE TESTS: HORSE SERUM	EYE TESTS: HORSE EP.	PASSIVE TRANSFERENCE SERUM SITES	INTERVAL IN DAYS: 1	2	3	4	5	6	QUANTITATIVE STUDIES: HORSE SERUM 1:100	H. S. 1:1,000	H. S. 1:10,000	HORSE EP. 0.001 MG. N PER C.C.	H. E. 0.0001 MG. N PER C.C.	H. E. 0.00001 MG. N PER C.C.
(5) L. S.	6	Toxin-Antitoxin at 18 months	Entire life	Positive	Marked	Moderate			R.	H. E. 0.01 Marked	→ Negative	H. S. 1:100 Negative									
									L.	H. S. 1:100 Marked	→ Negative	H. E. 0.01 Marked									
(6) M. R.	19	Toxin-Antitoxin 1921	Since infancy		Marked	Marked	Marked	Marked	R.	H. E. 0.5 Slight		→ Negative		H. S. 1:100 Marked		Reagins for Horse Serum			Reagins for Horse Ep.		
									L.	H. S. 1:100 Marked		→ Negative		H. E. .1 Negative							
(7) E. R.	12	Toxin-Anti at 6			Marked	Moderate			R.	H. E. 0.5 Moderate	±	→ Negative	H. S. 1:100 Moderate			present up to 1:1,000,000			present up to 0.01		
									L.	H. S. 1:100 Marked	Negative	→ Negative	H. E. 0.5 Negative								
(8) H. W.	15			Positive	Marked	Marked	Marked		R.	H. E. 0.01 Moderate		→ Slight		H. S. 1:100 Marked							
									L.	H. S. 1:100 Moderate		→ Negative		H. E. 0.01 Slight							

H. E.=Horse epithelium. H. D.=Horse dander. L.=left.
H. S.=Horse serum. R.=right.

occurred in the first five serums, whereas the exhaustion of the horse epithelium reagins after desensitization with horse serum occurred in only two instances, No. 6 and No. 7. In the remaining instance, No. 8, complete exhaustion of the reagins by either allergen failed; although the horse serum injection depressed slightly the reaction to horse epithelium, showing partial desensitization. Test tube neutralization with serums Nos. 3, 4 and 5 all checked the results with the ordinary method.

Quantitative Studies.—These were performed upon four of the serums. Serum No. 4 showed reagins in high concentration to horse epithelium as compared to horse serum; in No. 6 and No. 7 the concentration of horse serum reagins was extremely marked, a reaction with serum No. 6 being obtained even in a 1:1,000,000 dilution, while horse epithelium 0.01 gave only a slight reaction. This explains the fact that in both of these serums, the injection of horse epithelium failed to suppress the reagins to horse serum, whereas in serum No. 4, where the horse epithelium reagins were more marked, such suppression did occur.

The results of these experiments support, therefore, the conclusions to which Forster and Ratner and Gruehl came as a result of their experiments in the guinea pig: namely, that there must exist in horse dander extract two antigens, one peculiar to the dander itself, which for the sake of simplicity we might call the dander antigen, and the second one identical with horse serum but present in small amounts, possibly as a contaminant—this we can call the serum antigen which is identical with that present in horse serum. In explaining the results of the neutralization experiments, it seems evident that in the first five serums, where sensitiveness to horse epithelium seemed major, the extract contained enough of the serum antigen to desensitize the site and exhaust the horse serum reagins. In the last three serums, however, the concentration of horse serum reagins was too marked for this to take place, and exhaustion therefore did not occur.

So much then for the experimental aspects. From the foregoing data, it seems logical to assume that in the human being clinical sensitiveness could be manifested either singly to the serum or the dander antigen or to both antigens. In the latter instance such sensitivity might occur in greater degree to either of the antigens, depending upon the strength of the exciting factor.

This was borne out by a review of detailed clinical histories of atopic patients seen either in clinic or in private practice, in whom sensitiveness to either antigen existed. Thus in the following two instances sensitivity to horse dander alone was found:

CASE REPORTS

CASE 1.—J. M. C., aged six years, had had atopic eczema and asthma since infancy. Skin reaction and eye reaction were markedly positive for horse epithelium but negative to horse serum even in a 1:10 dilution. Serum reagins found in slight degree to horse epithelium only. Detailed inquiry into the history revealed the fact that since infancy the patient had been in frequent contact with horses without, however, any noticeable resultant untoward effects. Although the patient had received 3 injections of toxin-antitoxin at the age of five years, this apparently was insufficient to sensitize him to horse serum.

Even in the absence of known clinical symptoms in the patient, the positive skin test and eye test and serum reagins in an atopic patient are all evidence which indicate the presence of a constitutional or atopic sensitiveness to horse epithelium and not to horse serum.

CASE 2.—F. M., aged three years, had had allergic eczema at the age of one year, and recurrent attacks of asthma since the age of one and one-half years. No serum injections of any kind were received. Skin reaction was moderately positive to horse epithelium, and negative to horse serum. Inquiry into the history revealed the fact that from infancy to the age of one and one-half or more the patient had been in close contact with horse dander brought home by an uncle living in the same house and partly also because of frequent pony rides. The mother could not remember any symptoms resulting from this contact. However, on exposure to horse contact upon request, the child developed a generalized urticaria, lasting several hours.

This type of sensitiveness has not been considered unusual—the reverse situation, namely atopic sensitiveness to horse serum without coincident sensitiveness to horse epithelium, has hitherto been considered impossible. Such possibility, in my opinion, depends upon one's interpretation of the term atopic sensitiveness to horse serum. This is exemplified in the following case report:

CASE 3.—H. W. B., male, aged twenty-three, medical student, with a history of seasonal hay fever since 1933, showed a markedly positive skin and eye reaction and positive serum reagins to horse serum with entirely negative reactions to horse epithelium. To all intents and purposes, this would ordinarily be considered as an example of natural or atopic sensitiveness to horse serum. Inquiry into the history revealed the fact, however, that in 1922 the patient had two courses of toxin-antitoxin injections with no reaction to any of the injections. In 1931 following a prophylactic injection of tetanus antitoxin the patient developed both a severe accelerated and a delayed serum reaction, which lasted considerable time. Such a history makes it evident that the serum sensitiveness in this instance had been induced

or acquired as a result of this serum reaction and that it had established a permanent state of constitutional sensitiveness in an atopic individual.

These instances represent sensitivity to the single antigen. Combined sensitivity is a much more common phenomenon as is indicated by the 8 patients whose serums were obtained for the experimental study. Complete historical data unfortunately were not obtained upon all of these, but the data in two of these are of special interest.

CASE 4.—R. F. aged twelve years, had had asthma since 1925. Skin and eye tests were markedly positive to horse serum and horse epithelium. Serum reagins were present for both, but in much higher degree to horse epithelium than to horse serum. Since infancy the patient had been in frequent and close contact with ponies and horses, but not until 1928 were symptoms noted. At that time a short period of contact with a pony caused marked coryza, swelling of lids and lacrimation. Since that time, even distant exposure causes coryza. In November, 1927, the patient was taken to the Camden Municipal Hospital with an attack of diphtheria and given 20,000 units (7 c.c.) of horse serum diphtheria antitoxin intramuscularly with a preliminary dose of 1 minim given subcutaneously one hour previously. No serum reaction occurred. On December 5, 1929, he was readmitted with both scarlet fever and diphtheria and was given 40,000 units (14 c.c.) in similar fashion and with similar precautions. Again no serum rash was noted and no adrenalin given.

This history is quite remarkable in that in a patient who was a horse-asthmatic person a reinjection of a large amount of horse serum antitoxin after an interval of two years failed to produce any reaction whatsoever. Obviously this patient could not have been serum sensitive before either administration or serious reaction would have occurred. In this atopic or predisposed patient, prolonged contact with horses no doubt induced an exquisite degree of sensitiveness to the dander, whereas sensitivity to horse serum of a lesser degree occurred as a result of two injections of diphtheria antitoxin. Here again, the sensitiveness to horse serum, even though it occurs in an atopic individual, must be considered as of the acquired type.

CASE 5.—Female, M. R., aged nineteen years, complained of seasonal hay fever and asthma since June, 1930. Skin and eye reactions were found markedly positive to both horse serum and horse epithelium. In 1921 the patient had 3 injections of toxin-antitoxin without any ill effects. Since infancy she had been in almost constant contact with horses but had never noticed any symptoms until October, 1932, more than a year after the onset of her asthma. At this time after riding a horse for about ten minutes, the patient developed a marked coryza, asthma, and urticaria. Since then even slight contact with horsehair

provokes skin and eye irritation. Serum examination showed reagins to both horse serum and horse epithelium but in more marked concentration to horse serum. In this instance prolonged contact with horse dander produced clinical sensitivity only after the onset of the atopic illness, and even then it was more marked to the serum than to the dander. Whether the toxin-antitoxin injection can account for this is speculative, although in previous studies[7] I have shown that positive serum reactions to horse serum after toxin-antitoxin occurred more frequently in children of atopic families than in nonatopic.

CASE 6.—F. K., aged thirty-nine years, was skin and eye sensitive to horse serum and horse epithelium with positive reagins to both allergens, but in greater concentration to horse epithelium. Since early childhood the patient had been in almost constant contact with horses, but not until 1918 did he begin to evidence symptoms upon contact. This occurred coincidently with the onset of asthma, and since this time symptoms have occurred regularly after contact. Previous serum history was completely negative.

In this patient prolonged contact with horses finally induced in a susceptible individual sensitiveness not only to the dander antigen but likewise, in the absence of previous serum history, to the serum antigen as well. This would seem to bear out Ratner's suggestion of the induction of sensitiveness to both antigens by the respiratory route. It is interesting to note that in all of the patients sensitive to horse dander prolonged contact with horses was necessary to bring this out. This emphasizes the importance of contact in atopic hypersensitiveness as brought out by Coca.

SUMMARY

Clinical and experimental studies show that there exists in horse dander a small amount of antigen corresponding to that present in horse serum, and that in naturally sensitive or atopic patients sensitiveness may exist in varying degree to the dander antigen alone or to horse serum alone or to both of these antigens. Prolonged contact with horse dander by an individual predisposed to such sensitivity may result either in sensitiveness to the dander alone or in some instances to both horse dander and horse serum. Such sensitivity particularly to horse serum represents an instance of respiratory sensitization in the human being which is the counterpart of that shown to occur in the guinea pig in the experiments of Ratner and Gruehl. In patients sensitive to horse serum either alone or to horse dander as well, the possible influence of artificial sensitization by previous serum injection is important. From an academic standpoint it raises the question as to whether such instances are to be considered examples of true, natural or atopic hypersensitiveness or whether they

really belong to the acquired (or as some choose to call it) anaphylactic type, occurring in a predisposed individual. I personally am inclined toward the latter viewpoint.

Finally, from a practical standpoint, it is interesting to note that horse-asthmatic patients may withstand serum administration without any untoward effects. It is therefore advisable to test all allergic patients to horse serum both by skin and by eye test before dismissing them as unsuitable subjects for serum administration merely because of the existence of an allergic condition.

The author wishes to acknowledge grateful appreciation to Dr. Richard A. Kern for three of the serums used in this study and also to Mr. M. A. Brodsky, senior medical student at Temple University, for technical assistance in the passive transfer studies.

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1530 LOCUST STREET

DISCUSSION

DR. BRET RATNER, New York City.—It appears from Dr. Tuft's excellent presentation that experimental data carefully carried out will clarify many of the hypothetical speculations that invade our field. It is gratifying to think that Forster with precipitin test, we with the anaphylactic test, and now Tuft with the neutralization test have all found the identical situation to exist.

Atopy is defined as a strange disease, but as we go along we find the disease that we are dealing with is not quite so strange.

I think that from the very camp of the supporters of the hereditary hypothesis, if you scrutinize the literature carefully, you will find they themselves are bringing forth more and more evidence opposed to their original hypothesis.

The work of Spain and Cooke showed that the antibody artificially induced in rabbits could be artificially transferred to the human being.

Mrs. Grove has shown that with the blood of a hay fever patient sensitive to ragweed, she could induce a very definite passive sensitization in a monkey, which unquestionably links up the anaphylactic antibody and the Prausnitz-Küstner antibody.

In France, about twenty years ago a serum was introduced called Vallé serum for the treatment of tuberculosis, and a number of deaths and severe anaphylactic reactions occurred. Bernard, Debré, and

Porak, French investigators, took the matter in hand and quickly discovered that the individuals who got anaphylactic reactions were hippophagics, i.e., horse meat eaters, and they found precipitins in the blood of these individuals to horse meat and horse serum. So, there we have another interesting, definite proof that individuals acquire sensitivity to horse serum through the ingestion of horse meat.

In Russia they have reported marked reactions to antitoxins introduced in certain sections where the children were fed mare's milk.

The interesting instance that Dr. Tuft quotes of no reaction to the horse serum even though the individual was sensitive to horse dander, corroborates, as we have shown, that the common antigen in horse dander and horse serum is very small in amount, so that sometimes horse dander individuals can tolerate horse serum and not get reactions.

In testing out a case for horse serum hypersensitivity, as Dr. Tuft has rightly stated, a history of horse dander sensitivity is the paramount sensitivity which we must be fearful of. An allergic person can tolerate horse serum if he is not sensitive to horse dander.

Why Dr. Tuft still insists that these people are atopic in view of the excellent proof that we are dealing with an acquired sensitivity, is something that I still cannot ferret out.

I am delighted indeed to hear that these experimental facts that are being brought forth day by day seem to be dealing a lasting blow to the hereditary hypothesis, for we must gradually get to realize that what we are concerned with are anaphylaxis, antigen, antibody reactions, and allergy, and not atopy.

Dr. Leon Unger, Chicago.—I should like to ask Dr. Tuft what strength of serum he uses in the eye test.

As I take it [using the blackboard], Dr. Tuft means that horse dander equals S + D, i.e., the horse dander contains horse serum and horse dander factors. It seems analogous to the relationship between cottonseed and kapok. Cottonseed has two ingredients, the cottonseed factor and the kapok factor, and the kapok has only the kapok factor. Take a patient with the cottonseed and kapok sensitivity and run passive transfers and you can exhaust both of them. Take the kapok case and after you have exhausted the reaction to kapok, you can still get a positive test for cottonseed; after you have exhausted the cottonseed reaction, you will never get a reaction to kapok.

I think that is analogous to the horse dander and horse serum question.

Dr. Frank A. Simon, Boston.—I have recently been working on hypersensitiveness to horse serum and horse dander, and find, just as Dr. Tuft did in his work, that a passively sensitized skin site desensitized with horse dander is also desensitized to serum, but horse serum is not capable of desensitizing a passively sensitized skin site to horse dander. There is a factor in the dander which is not present in the serum.

Dr. Harry L. Huber, Chicago.—I am sorry that pediatricians and internists in general cannot hear papers of this sort.

Hooker, several years ago, showed that in those children who were given antitoxin or toxin-antitoxin, a certain percentage of them developed later on a sensitiveness to horses.

In an attempt to get some data on the apparent increase in the relative number of allergic patients, we have been asking our recently allergic patients if they had had antitoxin or toxin-antitoxin at any time, and it is surprising how large a proportion of these typically allergic patients had had at some time during their careers one of these horse serum preparations. Is it not possible that sensitizations may be initiated by the use of such preparations, especially in children?

DR. MARION B. SULZBERGER, New York City.—There are many important angles to this subject of Dr. Tuft's.

One of the important things is that most antigens, or allergens seem not to be monovalent, that is, contain not one but several active principles. I think that this is proved in the case of the horse dander; there is a horse dander specific and a horse serum specific component. One finds a similar polyvalence in many other allergens. For instance, in the case of ragweed, one can distinguish an oil allergen, the cause of eczematous dermatitis, and a "protein" fraction, the cause of hay fever. Furthermore, there may be a common allergen in ragweed and pyrethrum, in addition to a specific pyrethrum and a specific ragweed factor; that was brought out recently by Dr. Feinberg.

I feel that I must again take a stand on the almost eternal question which Dr. Ratner has brought up about atopy. There seems to me to be ground for thinking that there must be some confusion here, and that this confusion is largely based on misunderstanding. I am certain that the protagonists of the idea of familial and constitutional factors in certain types of hypersensitivity, called atopy, have never intended to imply that it was not necessary for an adequate contact to create the actual specific sensitivity or hypersensitivity in the atopic individual. A person may be as atopic as he can be, but if he does not come into contact with a certain allergen, he does not become sensitive to that allergen. In other words, the hypersensitivity itself is not hereditary, but the disposition to become specifically sensitized in a certain manner is hereditary. And, as Dr. Ratner has stated, the sensitization brought about by the contact with horse serum may be different in the atopic individual, from the sensitization which occurs through contact with the same horse antigen in nonatopic individuals.

If we do not adhere to the conception of atopy, how can we explain certain phenomena which are of daily clinical record? I do not think any of us here can deny today that if you find a case of real asthma, of real hay fever, or of real atopic dermatitis with skin reactions and reagins, you will be likely, upon questioning, to find a family history of these conditions. There is certainly a familial heaping of these hypersensitivities. There must be something running in that strain which predisposes that family to these forms of sensitivity.

This is in contrast to that which may be observed in the majority of other forms of sensitizations. Contact dermatitis, drug eruptions, mycotic and bacterial sensitizations do not evidence such familial occurrence, and we must therefore have a word to describe the familial occurrence of the predisposition to certain forms of hypersensitivity. The word which has been used to cover this phenomenon is "atopy" —and under atopy I understand only this phenomenon. I hope Dr. Coca will tell me if my understanding of the matter coincides with his.

DR. BRET RATNER, New York City.—I just want to add one word with reference to Dr. Sulzberger's discussion.

Dr. Sulzberger raised the question of atopy, and I want to ask him if an individual has atopy and never acquires a sensitivity, how does he know that patient is atopic?

DR. ROBERT WARD LAMSON, Los Angeles.—With reference to the case just mentioned by Dr. Tuft in which an atopy seemed proved and the patient was clinically sensitive to horse dandruff, but failed to react to an injection of horse serum, I should like to ask, are we justified in saying he is not or cannot be made sensitive to horse serum?

Reviews of the literature by Sturtevant, Weaver and others, on the subject to serum reactions, indicate that no more than 10 to 15 per cent of patients receiving 10 c.c. or less demonstrate any immediate or delayed response. When larger quantities of serum were employed, the percentage of reactions increased rapidly. Might it not be possible, therefore, that the patient would manifest an atopic or an allergic reaction if a larger quantity had been used?

DR. WARREN T. VAUGHAN, Richmond.—While this is getting off the subject, I will not enter the lists for or against the heredity of allergy, but I should like to record an interesting observation.

Our conclusions on the hereditary aspect of allergy are based upon studies of the frank, outspoken allergics, the 7 to 10 per cent of the population, as contrasted with those who are nonallergic. Take the 50 per cent of the population who have some minor, evanescent, allergic manifestation at one time or another but never sufficiently severe to require the patient's consulting a physician. I have compared their heredity with the remainder who have had no manifestation in the past, and I find equal inheritance in both groups.

DR. MILTON B. COHEN, Cleveland.—I had not intended to discuss this paper, but since we are off the subject and talking about the question of inheritance, atopy, terminology, and so forth, I should like to put in a word. That there is a group of patients who have a family history, nobody doubts. When we studied the reaction in human beings, histologically, to atopen or to allergen (for the benefit of Dr. Ratner), and histamine, we were certainly able to demonstrate a vast difference in the reactivity in the histologic picture, between normal individuals and those individuals who belonged in what we called a naturally sensitive group. Perhaps that may be only a quantitative one, but certainly it is a significant difference. I do not know how to explain it, but it exists.

DR. RACKEMANN.—I think you are right.

DR. GRAFTON TYLER BROWN, Washington, D. C.—With reference to what the previous discusser had to say, I was under the impression that toxoid contained no serum whatever, either sheep or horse, unless he refers to the blood in which the diphtheria bacilli were grown from which the toxins were obtained, which I think is rather remote.

DR. TUFT.—I rather expected that out of my presentation there would develop a discussion of the term atopy. I thought that possibly Dr. Coca would save me the necessity for defending this. However, Dr.

Sulzberger covered this in his discussion and I agree quite freely with his remarks in this connection.

I used the term "atopy" merely to indicate the constitutional predisposition to hypersensitiveness which by virtue of heredity is possessed by certain individuals. It is of course essential as has been stressed by Dr. Coca that contact of sufficient and prolonged character must occur in order to induce this sensitiveness or to bring out the reagins.

In answer to Dr. Huber concerning toxin-antitoxin, I covered this in a presentation several years ago on serum sensitiveness after toxin-antitoxin. I am now gathering data on its effect in allergic individuals.

I am not quite clear about Dr. Lamson's question as to the quantity that was necessary to induce the reaction.

Dr. Lamson.—Fourteen c.c. of the "anti" serum did not give reaction. Would you say 20 or 50 might not have? I got the impression you thought the patients did not have atopy or clinical hypersensitivity to horse serum.

Dr. Tuft.—The fact that the individual had asthma, and was sensitive to the horse dander indicated that individual was atopic. After the first injection of 7 c.c. if he had been atopically sensitive to horse serum, it would not have been possible to have given him even a minute fraction of this without inducing a reaction. He could not have been sensitive, therefore, at the time of the first injection. When he was given the second injection of 14 c.c. he certainly could not have been sensitive, or he would have reacted to even a fraction of this. Whether he would have reacted to 50 c.c. or 75 c.c. is something which I cannot answer definitely, but I believe that any reaction which might have occurred would have been of the delayed rather than of the immediate type. It is of course a well-known fact that in patients who are already sensitive to horse serum, the smallest amount, even a fraction of a minim, would induce a reaction to horse serum.

Dr. Brown answered the question on toxoid. As he stated, toxoid does not contain any horse serum.

ARSPHENAMINE HYPERSENSITIVENESS IN GUINEA PIGS

III. Experiments Demonstrating (A) the Regional Geographic Variability in Susceptibility to Sensitization; (B) the Chemical Specificity of the Hypersensitivity; and (C) the Variation in the Sensitizing Proclivities (Sensitization Index) of Different Brands*

Marion B. Sulzberger, M.D., New York, N. Y.
and
Frank A. Simon, M.D., Boston, Mass.

DESPITE the unquestionable advances in the modern prophylaxis and treatment of syphilis, this disease still remains among the most important which the medical profession is called upon to combat.

And, despite the unquestionable advances in modern antisyphilitic methods, which have come to include bismuth, malaria, tryparsamide, etc., the arsphenamines† are still the most important drugs used in the treatment of syphilis.

In countries in which arsphenamines are manufactured, there is governmental regulation of the testing of each factory lot of the drug, and each lot must be sold with its number affixed to the package. In the United States, each lot must be tested as to its therapeutic action in animals; and further the testing of the arsphenamines must determine the minimum lethal dose of each factory lot and thus insure a minimum of *toxicity* of the product. It is obvious that this procedure is of great value and should not be abandoned. However, the dosage of arsphenamine as today employed is so far below the minimum lethal dose for human beings that, from the practical viewpoint, the danger of actual *poisoning* is negligible. In other words, the therapeutic index of the arsphenamines is a very favorable one, and it is probable that no case of actual arsphenamine poisoning has been observed since the much larger doses first employed by Ehrlich and his school *(therapia magna sterilisans)* have been abandoned.

Our present dosage of arsphenamine has, indeed, almost entirely eliminated the danger of actual arsphenamine poisoning; nevertheless, although fortunately infrequently, grave sequelae and deaths still result from the administration of this drug. It is the consensus of opinion of those who have studied such sequelae that, while some may be attributed to other causes, they are due, in the main, to hyper-

*From the Department of Dermatology and Syphilis of the French Hospital, New York City; and from the Department of Anaphylaxis, Massachusetts General Hospital, Boston.

†In this paper, "arsphenamine," unless otherwise specifically stated, is employed in the generic sense, including old arsphenamine, neoarsphenamine, sulpharsphenamine, silver arsphenamine, etc.

sensitivity to arsphenamine; and that this hypersensitivity is, in many cases, the result of a sensitization process.

Most critical and excellent detailed summaries of the observations and arguments supporting this opinion are given by Stokes[1]; and, J. Klauder (cited by Stokes), by Mayer,[2] Jadassohn,[3] and Moore.[4] Among the manifestations of presumable or possible arsphenamine hypersensitivity, are the nitritoid crises, the early urticarial, morbilliform and scarlatiniform skin eruptions, the fixed skin eruptions, the purpuras, the acute yellow liver atrophies, encephalitis hemorrhagica, better called purpura cerebri, the leucopenic and agranulocytic symptom complexes, and, particularly, the late exfoliating and weeping dermatitis. The grave prognosis of many of these sequelae is well known, and physicians employing arsphenamine must be constantly on the alert for premonitory signs.

The susceptibility to these sensitizations depends upon various factors, many of which are, as yet, unknown. One calls that form of susceptibility to sensitization which is dependent upon the aberrant state, nature, or constitution of the individual patient, idiosyncrasy. This word is, of course, just a paraphrase which does not dispel ignorance concerning the actual causes underlying this state of abnormal, individual hypersensitivity.

Not only do patients vary in their individual susceptibility to arsphenamine sensitization, but it is acknowledged that the various arsphenamines and, more strangely still, even different factory lots of the very same arsphenamine, may vary as to the probability of their producing such sensitizations. There are many examples showing that, for some as yet unknown reason, a certain lot coming from a certain factory will, unlike the other supposedly identical lots coming from the same factory, suddenly cause a series of cases of acute yellow liver atrophy, or of sensitization dermatitides, or of nitritoid crises (Moore,[4] Stokes,[1] Schamberg [quoted by Stokes[1]], Wile,[5] and J. Jadassohn[6]).

Several years ago, one of us observed a series of cases of exfoliating dermatitis, produced in quick succession by a certain factory lot of neoarsphenamine. The pharmaceutic firm manufacturing this arsphenamine, which had heretofore supplied a product in all ways satisfactory, was notified of this occurrence. The offending lot, which had apparently been manufactured in exactly the same way as the preceding ones and had passed the same laboratory tests, was withheld from the market.*

*Certain European governments, recognizing that the possible deleterious effects of an arsphenamine cannot always be discovered by toxicity tests in the laboratory, have made it compulsory to have each factory lot, not only *laboratory tested*, but also *clinically tested* before it may be marketed. Three public governmental hospitals receive each new lot for testing. The particular lot of arsphenamine can be marketed only after sufficient tests on clinical material have proved it to be safe (i.e., not an unusually strong sensitizer, "idiosyncrasiogen," "allergen"). It was at such an official governmental testing clinic that the above mentioned lot causing dermatitides was discovered.

Recently, in private practice, a new brand of neoarsphenamine was employed in treating three syphilitic persons who had been under treatment with another neoarsphenamine for some time and had had no ill effects from the injections. The equivalent dose of the new lot caused headaches, nausea and vomiting, and intestinal spasms in all three patients; and, in one patient, the crisis was so severe that the condition became alarming.

The particular lots of neoarsphenamine which caused these sudden, startling frequencies of untoward reactions had presumably all been standardized as far as toxicity was concerned, according to governmental regulations; and were presumably no more *poisonous* than the other lots turned out by the same factories.

It is obvious that there must be an unknown attribute (Cf., X-factor of Schamberg [quoted by Stokes[1]], and of Harrison[7] in liver damage) of certain preparations of arsphenamine, which renders them more dangerous, more likely to cause sequelae of certain types, more likely to sensitize. This opinion has been expressed by J. Jadassohn,[6] Br. Bloch, and others.[1]

Since the fundamental experiments in arsphenamine sensitizations, which were carried out in J. Jadassohn's clinic by Frei[8] in 1928 in both man and animals, investigators have had at their disposal: (a) a method for studying the predisposing idiosyncratic state which renders the individual prone to sensitization; and (b) a method for determining, in animals, which arsphenamine and which batch of arsphenamine is the most likely to be a potential sensitizer.

In experiments with arsphenamine sensitizations in guinea pigs, Mayer and Sulzberger[9, 10] found that: (a) guinea pigs could be sensitized with great ease and regularity in Breslau, Germany; (b) with the same technic it was practically impossible to sensitize New York guinea pigs in New York City; (c) the susceptibility or refractoriness to sensitization was not a racial characteristic of the guinea pigs, for Sulzberger and Mayer, in unpublished experiments, found that 35 New York guinea pigs brought to Breslau and to Zürich, Switzerland, could (themselves and their progeny) be sensitized with the same ease and in the same percentage as the indigenous animals; (d) the drug used was not the variable factor (the same brand was used both in New York and in Europe); (e) the European sensitizations were susceptible to seasonal influences, a greater percentage of animals becoming sensitized at the end of the winter and a lesser percentage at the end of the summer; (f) the addition or absence of green fodder influenced the percentage of sensitizations in Europe, the green fodder having an inhibiting influence.

Many of these observations in arsphenamine sensitization also apply to sensitization with ursol (paraphenylendiamine[9, 10]) and with

tubercle bacilli and tuberculin (von Engel[11]) and would, therefore, seem to have general significance. The experiments referred to have, to date, been made on many hundreds of guinea pigs over a period of more than five years.

In June, 1933, it was decided that an attempt should be made to sensitize guinea pigs to arsphenamine in Boston. This decision was prompted by the following considerations: first, the knowledge that it had heretofore been impossible to sensitize guinea pigs to arsphenamine in New York City;[10] second, the variability in susceptibility to sensitization apparently dependent upon the geographic location of the experiment (in addition to the variations mentioned, between Breslau, Zürich, and New York,[10] Kaplun and Moreinis,[12] were successful in sensitizing and desensitizing guinea pigs with arsphenamine in Odessa). Third, Simon reported that he had been able to sensitize Boston guinea pigs in Boston with poison ivy extracts, while Spain[13] and his coworkers had been unable to do so in New York on New York guinea pigs; moreover, Simon could sensitize Boston guinea pigs in Boston with ursol, just as in the Breslau experiments of Mayer.[9] Fourth, it had been found in Breslau that the laws governing susceptibility to eczematous sensitization with ursol were analogous to those governing the arsphenamine sensitizations.[9] Fifth, in the event of successful arsphenamine sensitizations in Boston, the proximity of Boston to New York, as compared to the distance between New York and Breslau, would make it more feasible to study the environmental and geographic factors which seemed to exert such a profound influence on the susceptibility of the guinea pigs to sensitization in these experiments. Sixth, the discovery of the factors influencing sensitizations in guinea pigs might prove of value: (a) in influencing sensitizations in man; (b) in enabling investigators to produce hypersensitivity in guinea pigs with a predictable regularity; so that drugs, and especially various arsphenamines, could be standardized on animals, not only as heretofore for their *toxicity*, but also for their potential *sensitizing* action as well. Then, the lots, before being employed on human patients, would be regulated not only as far as their minimum lethal dose is concerned, but also for what we might call their "sensitization index" (probability of producing sensitization).

Before reporting upon the results of experiments which were undertaken as a result of the above considerations, we must not neglect to mention two points—however obvious they may be. First, the actual causes of arsphenamine sequelae in man are unknown, and sensitization alone cannot account for all the phenomena observed. There are certainly other factors which may play a leading or contributory part in the causation of arsphenamine by-effects. Second, information gained concerning sensitization with arsphenamine in guinea pigs is

not necessarily directly applicable to *arsphenamine sensitization in man.* However, the same factor of error may exist in the toxicity experiments and therapeutic experiments on animals, which are now nevertheless the official and accepted and obligatory criteria of the value of arsphenamine preparations. Just as animal tests have proved useful in establishing a therapeutic index for the arsphenamines, just so it is more than possible that *sensitization assays on animals may serve as an indicator of the sensitizing propensities of a drug in man.*

EXPERIMENT I

During the summer of 1933, 11 guinea pigs were treated with neoarsphenamine in Boston. The preparation employed was "Neodiarsenol." The technic was identical with that first used by W. Frei in Breslau, and by Sulzberger and Mayer[8] in Breslau, Zürich, and New York.

Healthy guinea pigs weighing from 200 to 400 grams were employed. The neoarsphenamine solution was prepared by first dissolving 0.15 grams in 10 c.c. of distilled water, and then adding 90 c.c. of a physiologic solution of sodium chloride. One-tenth c.c. of this solution (containing 0.00015 grams of arsphenamine) was injected, as superficially as possible, into the previously shaved or depilated skin area on the flank of the guinea pig. The same dose was used for sensitizing and for testing four weeks later. The test injection was given at a point distant from the first injection (on the opposite flank, in the majority of instances).

The sensitization manifested itself in the same manner as in Europe: (a) by a flare-up of the original site, six or more days after the injection, which occurred in a certain percentage of the sensitized animals; and (b) by a markedly stronger reaction to the second injection in the sensitized animal, documenting itself in an erythema and infiltration (and sometimes central necrosis) appearing within twenty-four hours after the administration of the drug.[14]

Of the 11 guinea pigs in this first Boston experiment, 6 became sensitized. This preliminary experiment demonstrated that *Boston guinea pigs in Boston could be sensitized to neoarsphenamine, with a regularity comparable to that achieved in Europe. They differed in this respect from the guinea pigs previously and similarly treated in New York.*

EXPERIMENT II

An attempt was made to study the factors which might account for the difference in susceptibility to sensitization in Boston and New York as stated, New York guinea pigs and their progeny when transported to Europe had become susceptible to sensitization. It could, therefore, be concluded that environmental factors and not racial ones

determined the refractoriness of the New York animals. In this preliminary experiment, in which both fodder and guinea pigs were transferred from New York to Boston, and vice versa, *no definite conclusion* could be reached *regarding the nature of the environmental factors.** The Boston fodder consisted of hay, oats, and carrots, all purchased in Boston. The New York fodder consisted of hay, turnips, and Irish potatoes, boiled and made into a mash by adding equal parts of wheat-bran and oats. The New York vegetables and hay were purchased in New York. The animals gained weight on both of these diets. They were placed upon these diets both in Boston and in New York on November 28, 1933. The first injection of arsphenamine was given in both cities on December 13, 1933. There was thus a period of pre-feeding of fifteen days' duration. The test infection was given in Boston and in New York on January 17, 1934. There was thus an interval of thirty-five days between first and second injections. The individual series of guinea pigs on each type of fodder in each city were too small, and the time of pre-feeding was too short to allow of any conclusion as to the effect of diet and environment. It can only be stated that, of the 48 guinea pigs in the experiment, the 7 New York guinea pigs on New York fodder in New York were all refractory to sensitization; and of the 6 Boston guinea pigs on Boston fodder in Boston, 4 became sensitized.

TABLE I

ORIGIN OF ANIMALS	NUMBER OF ANIMALS	NUMBER BECOMING HYPERSENSITIVE	APPROXIMATE PERCENTAGE
Boston guinea pigs	34	21	62%
New York guinea pigs	14	3	21%
Place of experimentation			
Guinea pigs in Boston	29	21	72%
Guinea pigs in New York	19	3	16%

For the rest, the results, as shown in Table I, simply indicated that Boston animals were, on the whole, much more easily sensitized than New York animals; and that it was, in general, easier to sensitize guinea pigs in Boston than in New York, regardless of the place of origin of the animal.†

In order to be certain that there would be a minimum of unknown variable factors in this experiment, we employed, both in Boston and in New York, the same factory lot of neoarsphenamine—neodiarsenol; and the same make of glass syringes, of glassware, and of cannulas. We also employed, in both cities, the Boston distilled water and Boston saline solution.

*We, too, have considered the possibility that latent infection might be a factor in predisposing guinea pigs to arsphenamine sensitization, just as Stokes[1, 15] and his coworkers have suggested in the human sensitizations. No experimentors have as yet attempted to study this possibility.

†This experiment can be regarded only as preliminary to a more extensive one along the same lines, which is now being planned. It will, of course, be necessary to have a longer period of pre-feeding and a much larger number of guinea pigs, so that there will be 8 groups, each one with a number of animals sufficient to obviate the error of small series.

EXPERIMENT III

The following experiment was carried out in Boston on 6 guinea pigs previously sensitized to neoarsphenamine and proved to be skin hypersensitive to intradermal reinjections of the drug; and on 2 normal (not previously sensitized) control animals.

One-tenth c.c. of a solution of the following arsenicals was injected intradermally: (1) neoarsphenamine (neodiarsenol) (trivalent organic

TABLE II

Compound	Formula	Valence Arsenic	Per cent Arsenic	Amount Injected Equivalent	Dose of Arsenic in Mg	Result of Skin Test in m.m. Normal Pigs	Result of Skin Test in m.m. Pigs sensitized to Neo.
Neoarsphenamine		3	20	0.15	0.03	°	⑥ ⑦ 12 ③ ⑤
Arsphenamine		3	34	0.09	0.03	④ ⑦ ⑤ 10 11 ⑥ ⑦	
Trypars-emide		5	25	0.12	0.03	° ° ° ° ° ° °	
Sodium Cacodylate		5	35	0.086	0.03	° ° ° ° ° ° °	
Sodium Arsenate	$NaH_2AsO_4 \cdot 7H_2O$	5	24	0.12	0.03	° ° ° ° ° ° °	
Potassium Arsenite	K_3AsO_3 Made up from $As_2O_3 + H_2O + KHCO_3$	3	75 (in As_2O_3)	004	0.03	⑥ 11 ⑥ ⑤ ⑥ 9 9 9	

aromatic arsenical); (2) old arsphenamine (neutralized) (trivalent organic aromatic arsenical); (3) tryparsamide (pentavalent organic aromatic arsenical); (4) sodium cacodylate (pentavalent organic aliphatic arsenical); (5) sodium arsenate (pentavalent inorganic arsenical); (6) potassium arsenite (trivalent inorganic arsenical).

The dosage was reduced to an equivalent common quantity of the element arsenic in each injection, i.e., each injection contained 0.03 mg. of arsenic.

The results of this experiment are set forth in Table II. As will be seen, the guinea pigs sensitized with neoarsphenamine were sensitive to this drug itself and to the old arsphenamine, but not to the tryparsamide, sodium cacodylate, or sodium arsenate. Furthermore, as can be seen in Table II, the potassium arsenite in this dosage proved irritating to the skin both of the neoarsphenamine sensitized animals and of the controls.

As the irritating effect of the potassium arsenite prevented the decision as to whether or not the animals were hypersensitive to this arsenical, it was decided to compare the effect of one-tenth the original dosage of potassium arsenite with one-tenth the original dosage of neoarsphenamine, on 9 guinea pigs known to be hypersensitive to neoarsphenamine and on 3 controls. The results of this experiment are set forth in Table III. They show that, while the potassium arsenite was still slightly irritating to the skin of the guinea pigs, in both control and sensitized animals, there was no evidence of a hypersensitivity to this drug in the neoarsphenamine hypersensitive animals.

TABLE III

COMPOUND	DOSE OF ARSENIC	CONTROLS			MEASUREMENTS IN MM. 24 HOURS PIGS SENSITIZED TO NEOARSPHENAMINE								
		1	2	3	1	2	3	4	5	6	7	8	9
Neoarsphenamine	0.03	2	4	3	7	10	5	10	15	6	15	10	9
Neoarsphenamine	0.003	2	5	4	6	8	2	4	7	7	10	6	4
$As_2O_3 + KHCO_3$	0.03	10	12	10	10	10	15	8	9	7	12	9	15
$As_2O_3 + KHCO_3$	0.003	2	5	5	2	3	2	4	0	6	6	5	8

It is to be concluded that the sensitization to neoarsphenamine, as produced under the conditions of our experiments and as brought to light by our technic in testing, is directed toward the whole or a part of the chemical complex of the organic aromatic trivalent arsenicals, *old arsphenamine* and *neoarsphenamine*. *It is not a hypersensitivity to arsenic*, which has been produced.*

*It is a question whether such hypersensitivity might not be directed toward benzol derivatives in general, or against diazo-like compounds as a group. In a few animals tested, intradermal injections of *pyramidon* solutions in guinea pigs hypersensitive to *neoarsphenamine* did not elicit reactions. Further study along these lines is indicated and may bring to light certain group relationships of the hypersensitivities. These studies are particularly indicated in view of the fact that the arsenobenzols, pyramidon, and certain other benzol derivatives are clinically known to cause fixed drug eruptions of very similar appearance, and that the drugs of this group are now incriminated as possible causes of granulocytopenia. (Madison and Squier.[16])

These results showing the specificity of the animal sensitizations to neoarsphenamine are, in general, in harmony with those of investigations in human arsphenamine dermatitides. Many, and perhaps most, cases of arsphenamine dermatitis are not sensitizations to arsenic, but *sensitizations to specific organic arsenic compounds.* The clinical characteristics of arsphenamine dermatitis are frequently different from those of arsenic dermatitis. For example, in arsphenamine dermatitis there is a general lack of subsequent keratoses and precanceroses, etc. Skin tests on human beings bear out this differentiation. Frei and Mayer[17] report experiments with skin tests in arsphenamine dermatitides, which show a specificity analogous to that which we have found in animals. These authors also give an excellent summary of previous similar experiments in arsphenamine sensitizations in human beings.

It is undoubtedly true that cases of polyvalent hypersensitivity may be found in patients suffering from arsphenamine dermatitis. Some of these patients are, therefore, sensitive not only to arsenobenzol, but also to arsenic, sometimes in all possible forms, and even also to other heavy metals such as bismuth and mercury (J. Jadassohn, S. K. Rosenthal, H. Biberstein, quoted in Ref. 17). Nevertheless, the most frequent occurrence in human arsphenamine hypersensitivity seems to be that the patient is sensitized specifically to the arsenobenzol complex, and reacts to this alone. It is, therefore, an error to assume that *arsphenamine hypersensitivity* is identical with *arsenic hypersensitivity;* and loosely to refer to arsphenamine dermatitis and to arsphenamine sensitization as "arsenic sensitivity" and "arsenic dermatitis."

EXPERIMENT IV

The following experiment was carried out at our suggestion by Drs. George S. Williamson and C. A. Morrell in Ottawa, Ont. We are indebted to them for their kind permission to incorporate their results in this paper.

In the winter of 1934, 19 guinea pigs of the stock of an Ottawa laboratory, from a strain which has been perpetuated without admixture for seven years, were sensitized with two different brands of neoarsphenamine. The technic employed was that used by us in New York and Boston.*

The results of this experiment are set forth in Table IV. As will be seen, 100 per cent of the guinea pigs became sensitized. However,

*The Ottawa fodder was as follows:

1. *Daily* throughout the year: (a) Blatchford's XL mixed scratch grain (percentages of cracked corn, wheat and oats); (b) carrots; (c) hay.
2. Daily *when in season:* alfalfa and kale.
3. *Once a week* throughout the year, rat mixture made as follows: Whole wheat, 10.8 kg.; rolled oats, 10.4 kg.; cracked corn, 10 kg.; casein (commercial), 2 kg.; lard, 4.4 kg.; sodium chloride, 200 gm.; calcium carbonate, 200 gm.; sodium iodide, 8 gm.; yeast (dried), 100 gm.; cod liver oil, 400 c.c.

N.B. This diet was fed ad lib.

TABLE IV

GROUP I. { MATERIAL BRAND "G" OF NEOARSPHENAMINE.
SOLUTION—0.15 GM. DISSOLVED IN 10 C.C. AQ. DIST. DILUTED TO 100 C.C. with FRESH 0.85%—NaCl SOLUTION.
DAYS DOSE—0.1. C.C. FOR EVERY 200 GM. BODY WEIGHT.

PIG NO.	AGE IN DAYS	WT. IN GM.	NEO I.D	1	2	3	4	5	6	7	8	9	10	13	17	20	30	NEO 0.1 I.D	1	2	3	4	8	12	17
33	79	400	0.2	(+)	(+)	((+))	((+))	⊖	⊖	⊖	⊖	(+)	+	+	N (+)	N ((+))	SC	0.1	††	††	††	N +	N (+)	N	SC
37	63	328	0.16	(+)	(+)	((+))	⊖	⊖	⊖	⊖	⊖	((+))	(+)	+	N (+)	N ((+))	SC	0.1	††	††	††	N +	N (+)	N	SC
71	101	440	0.22	(+)	(+)	((+))	((+))	⊖	⊖	⊖	⊖	((+))	(+)	+	N (+)	N ((+))	SC	0.1	††	††	††	N +	N (+)	N	SC
81	87	368	0.18	(+)	(+)	((+))	⊖	⊖	(+)	+	+	+	N +	N ††	N +	N (+)	SC	0.1	††	††	††	N +	N (+)	N	SC
101	64	360	0.18	(+)	(+)	((+))	⊖	⊖	⊖	⊖	((+))	(+)	+	N ††	N +	N ((+))	SC	0.1	††	††	††	N +	N (+)	N	SC
104	63	332	0.16	(+)	(+)	((+))	⊖	⊖	⊖	⊖	⊖	⊖	⊖	⊖	⊖	⊖	⊖	0.1	††	††	††	N +	N (+)	N	SC
105	63	276	0.14	(+)	(+)	((+))	⊖	⊖	⊖	⊖	((+))	(+)	+	+	N (+)	N ((+))	SC	0.1	††	††	††	N +	N (+)	N	SC
133	71	320	0.16	(+)	(+)	((+))	⊖	⊖	⊖	⊖	(+)	+	+	N +	N (+)	N (+)	SC	0.1	††	††	††	N +	N (+)	N	SC
135	66	304	0.15	(+)	(+)	((+))	⊖	⊖	⊖	⊖	⊖	((+))	(+)	+	N (+)	N ((+))	SC	0.1	†††	†††	††	N ††	N +	N	SC
200	64	368	0.18	(+)	(+)	((+))	((+))	⊖	⊖	⊖	⊖	((+))	(+)	+	DIED										
Date			Jan. 2/34												Jan. 19/34		Feb. 1/34	Mar. 9/34							Mar. 26/34

GROUP II. { MATERIAL BRAND "A" OF NEOARSPHENAMINE. / SOLUTION / DOSE } AS IN GROUP I.

DAYS

PIG NO.	AGE IN DAYS	WT. IN GM.	NEO I.D	1	2	3	4	5	6	7	8	9	10	13	17	20	30	NEO 0.1 I.D	1	2	3	4	8	12	17
40	97	452	0.22	(+)	(+)	((+))	⊖	⊖	⊖	(+)	+	+	N ++	N ++	N +	N (+)	SC	0.1	+++	+++	N +++	N ++	N +	N	SC
68	69	392	0.19	(+)	(+)	((+))	⊖	⊖	(+)	+	+	+	N +++	N +++	N ++	N +	SC	0.1	+++	+++	N +++	N ++	N ++	N	SC
69	91	436	0.22	(+)	(+)	((+))	((+))	⊖	(+)	+	+	++	N +++	N ++	N ++	N +	SC	0.1	+++	+++	N ++	N +	N (+)	N	SC
99	63	364	0.18	(+)	(+)	((+))	⊖	⊖	(+)	+	++	++	N +++	N ++	N ++	N +	SC	0.1	+++	+++	N +++	N ++	N +	N	SC
102	64	300	0.15	(+)	(+)	((+))	⊖	⊖	⊖	⊖	⊖	+	N +	N +	N (+)	N ((+))	SC	0.1	++	++	N ++	N +	N (+)	N	SC
106	63	264	0.13	(+)	(+)	((+))	((+))	⊖	⊖	0	+	++	N ++	N ++	N (+)	N ((+))	SC	0.1	+++	+++	N +++	N +++	N ++	N	SC
129	48	340	0.17	(+)	(+)	((+))	⊖	⊖	⊖	⊖	⊖	+	+	N +	N (+)	N ((+))	SC	0.1	++	++	N ++	N +	N (+)	N	SC
136	64	304	0.15	(+)	(+)	((+))	((+))	⊖	(+)	+	+	++	N +++	N +++	N ++	N ++	SC	0.1	+++	+++	N ++	N ++	N +	N	SC
137	64	332	0.16	(+)	(+)	((+))	⊖	⊖	⊖	⊖	+	+	N +	N ++	N +	N (+)	SC	0.1	++	++	N ++	N +	N (+)	N	SC
			Jan. 2/34															Mar. 9/34							Mar. 26/34

((+)) —Quarter of Split Pea Size.
(+) —Half of Split Pea Size.
\+ —Split Pea Size.

++—Bean Size.
+++—Dime-to-Nickel Size.
N—Necrosis.
SC—Scar.

the sensitizations varied in that the animals in Group II sensitized with one make of neoarsphenamine became more highly hypersensitive than those in Group I sensitized with another brand.

The flare-ups occurred earlier and were more marked in Group II than in Group I. The necrotic areas and subsequent scars were also more evident in the second group. The response to the second injection was distinctly stronger in Group II. This would suggest that *there is a difference in the sensitizing action (sensitization index) of the two brands of neoarsphenamine employed*, for the only variable factor in this experiment was the different brand used in each group.

Experiment IV seems to bring to light a difference in sensitizing power (sensitization index) between two different brands of neoarsphenamine. In our opinion, it would be of paramount interest to ascertain whether one of these brands of arsphenamine, more than the other, displayed a tendency to produce sensitization and unpleasant sequelae in human beings; and, whether this greater tendency toward human sensitization corresponded to the higher "sensitization index" as determined in guinea pigs. Unfortunately, no such clinical data are available.

The possible practical importance of a continuation and further development of such experiments is apparent. It is to be hoped that there will be opportunities for the correlation of the findings of the "sensitization index" of a certain lot of a drug in animals, with the clinical observations of the proclivities of this lot to produce sensitization sequelae in man. The establishment of the sensitization index in animals may eventually prove to be a laboratory procedure capable of detecting and preventing the distribution of such lots as may be particularly likely to produce human sensitization and undesirable sequelae. When this will have been achieved, such sensitization tests should become obligatory; of course, not to supplant, but to supplement the toxicity tests now legally required.

SUMMARY

1. Guinea pigs can be sensitized by intradermal injection of neoarsphenamine, in Boston, Mass.; Breslau, Germany; Zürich, Switzerland; Odessa, Russia; and Ottawa, Ontario. This sensitization can be achieved with relative regularity and ease.

2. New York guinea pigs in New York and on New York fodder have proved to be difficult and, in some series, impossible to sensitize, with the same technic and employing the same instruments, water, and brand of neoarsphenamine as used successfully in Boston. The cause or causes of this variation in susceptibility to sensitization are not racial or constitutional, for New York guinea pigs (35) when transported to Breslau and to Zürich could there be sensitized with

the same regularity as the indigenous animals. It has been impossible, as yet, to discover the environmental factor or factors responsible for the variability in susceptibility to sensitization.

3. The hypersensitivity experimentally induced in guinea pigs by intradermal injection of neoarsphenamine is apparently specific to the arsenobenzol complex. It is not a hypersensitivity to other pentavalent and trivalent organic and inorganic arsenicals; *and it is not identical with hypersensitivity to the element arsenic.* This corresponds with the findings of specificity of hypersensitivity in many cases of arsphenamine dermatitis in man.

4. In Ottawa, Williamson and Morrell have shown that guinea pigs sensitized with one brand of neoarsphenamine became more strongly hypersensitive than those sensitized with another brand, all other conditions of experimentation being identical. This suggests that different lots of neoarsphenamine may vary in their sensitizing proclivities (sensitization index) in man as well as in animals. The sensitization index would seem to be independent, to a great degree, of the actual toxicity. For this reason, it may often be impossible to discover the "sensitization index" by means of the usual toxicity test. In the case of drugs, such as arsphenamine, in which the undesirable sequelae are apparently in great part due to sensitizing action, it would seem indicated to establish, in animals and before general distribution, the sensitization index of each factory lot; and thus detect and prevent the clinical employment of those lots which are the strongest sensitizers in animals. Continuation and further development of sensitization experiments in animals and their correlation with clinical observations are necessary. Only thus can be determined whether the weeding out of lots of arsphenamine with high "sensitization index" in guinea pigs will also reduce the incidence of human sensitizations.

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DISCUSSION

DR. JOSEPH L. MILLER, Chicago.—At St. Luke's Hospital, in the outpatient department, one nurse for the past two years has prepared neoarsphenamine which is given to patients. These patients are divided into groups, and twice a week treatment is given. Within a month or two after doing this work the nurse developed an asthma which she had never had before and has had attacks of asthma ever since; so the interne thought he would make a skin test of neoarsphenamine, and there was marked reaction, nausea and vomiting, and immediately afterward she had an attack of asthma. I wonder whether that is a common thing among people preparing neoarsphenamine.

DR. WILLIAM L. BEECHER, Chicago.—I want to ask Dr. Sulzberger that if the guinea pigs that were sensitive in Boston, were taken to New York, was there any variation in the sensitivity after they were taken there; whether it was location or food there. I think that might have a bearing upon the possibilities of other sensitizations of people going from one climate to another.

DR. ALBERT H. ROWE, Oakland, Calif.—An observation that I have recorded previously over a period of years is the beneficial effect that dry and particularly high regions have on symptoms due to food sensitization. Such symptoms are especially liable to occur near the seacoast, and particularly in the area around San Francisco Bay. It is my custom to tell patients that they will be able to tolerate foods better in such regions, and that they will have to be especially careful of their diets when they return to the sea level. It has also been evident that symptoms due to food sensitization are less liable to occur during June, July, and August, and that exacerbation of the results of such allergy is quite possible as September and October come around.

Similar observations have been recorded by investigators in Germany, and also by Van Leuwen. In Europe an endeavor has been made to determine the fundamental cause of this seasonal effect. The effect of the ionization of air, humidity, and barometric pressure has been considered; but no definite satisfactory solution has been discovered. In my own work, I have assured myself over a period of years that bronchial asthma and perennial hay fever are especially liable to be severe during the same months that apparently affect pa-

tients in the low regions of Europe. I am very sure that humidity is not the explanation, and I am inclined to favor some influence that barometric pressure might have on the susceptibility of tissues to such allergic reactions.

I should be interested to know whether Dr. Sulzberger has ever considered the possibility of determining whether it is possible to desensitize his Boston or New York guinea pigs in one season rather than in another season.

Dr. George L. Waldbott, Detroit.—I wonder whether Dr. Sulzberger's determination of the sensitizing index is really of great importance. To me, the sensitizing property of a drug does not seem to depend so much on the type of the drug administered as it does on other factors. Any drug is likely to produce an allergic reaction. The important question, however, is whether or not there had been previous contact with this drug, how large the dose is, and what the time interval was between the previous contact. Those drugs which are most frequently taken by the public, such as aspirin, quinine, and phenolphthalein, seem to cause most trouble.

I have had occasion to study an autopsy of a case of exfoliative dermatitis, following the administration of arsphenamine, and am familiar with some recent reports in the literature. This condition, it seems to me, is very closely related to serum sickness. It occurs between five and twelve days after the injection of arsphenamine, and there are other clinical and pathologic features which both conditions have in common. I wonder whether Dr. Sulzberger, with his large clinical experience, could enlarge on the various manifestations of drug sensitivity, and draw a parallel with the lesions that occur with other antigens. Any allergic lesion, I believe, may arise as the result of the administration of a drug. Aside from the common cutaneous lesions, I know of cases of serum sickness, allergic shock, asthma, vasomotor rhinitis, gastrointestional allergy which were produced by drugs. There is, of course, a well-established affinity of certain drugs to certain tissues which may probably account for the specific involvement of particular organs by given drugs.

Dr. Bret Ratner, New York City—I should like to ask Dr. Sulzberger whether the use of insecticides containing arsenic may have some bearing on the difference in the animals' reactions.

Dr. Harry L. Huber, Chicago.—Dr. Sulzberger spoke of the difference in Boston and New York guinea pigs, but he did not mention the differences in the diets of these pigs. He also spoke of the exportation of the pigs, but I do not recall that he spoke of the exportation of the foods.

Those who have done aphylactic experiments in guinea pigs know that different groups of pigs vary greatly in reactibility. In some cases the food of the pigs has been shown to account for this variability.

Several years ago while working on a copper problem I made some analyses of foods given to guinea pigs, and was very much amazed at the large variation in the amount of copper that was found in spinach and in some of the other foods. Other constituents are probably just as variable. I think sometimes that we do not pay enough

attention to the food that is given these experimental animals. We know of instances where cockroaches around the laboratory have produced irregularities in the results of vitamin experimentation.

DR. BEN RAPPAPORT, Chicago.—The question of the difference in guinea pigs in the different cities is probably (to me, at least) more interesting than the work on the arsphenamines. I wonder whether the work of Dr. William F. Petersen of Chicago, on the effect of weather changes on disease might not (though it may sound far fetched) offer some means of explanation.

It would be very interesting to study the same groups of guinea pigs from the same litters, transferred to a place where there are sudden weather changes, as in Chicago, or farther north, and a similar group farther south, where the changes are relatively slight and not at all sudden.

Many of you probably are familiar with Dr. Petersen's work, especially as it relates to the migration of the Negro from the South, where he has become accustomed to the climate, and the diseases that he develops, many of them allergic, when he moves farther north.

Also it will be interesting to know how long the guinea pigs exported from New York to Russia, I believe it was, remained there before experimental work was started on them.

DR. JOSEPH HARKAVY, New York City.—I think Dr. Sulzberger's work has a great many points which are important for the student of anthropology as well as the allergist. His results in animals may explain some of the variations in reactions of peoples in different countries and various localities. We have to take into consideration the influence of the chemistry of the soil on the qualitative differences in the vegetables grown, and in turn the effects of ingestion of such foods on human as well as animal sensitization. That chemical differences as well as relationships are important is seen in a general way in everyday practice and has been stressed by Vaughan, Landsteiner, and others.

DR. SULZBERGER.—I was interested in Dr. Miller's story of the nurse who prepared arsphenamine solutions. I have seen a practically identical case which was referred to me from the New York State Syphilis Clinic in Albany. Dr. Pfeiffer had a nurse who prepared the solutions and who had asthmatic attacks, gastrointestinal allergy, and skin reactions from the arsphenamine with which she worked. She was once on the point of being operated on for appendicitis, which was later proved to have been a reaction of idiosyncrasy to the drug. She gave a wheal reaction with pseudopods to scratch tests with arsphenamine. This was so large and alarming that we had to give her adrenalin and block it off. She had no reagins to arsphenamine.

As to the question whether we transferred the already sensitive guinea pigs from Boston to New York: we have not gotten that far yet. We are trying to find out whether the transfer of normal animals from Boston to New York will make them lose their disposition to become sensitized.

I am interested in what Dr. Rowe has said. That is our own impression of the situation here. The actual content of foods is, as Dr. Harkavy, too, has said, certainly more varied than was formerly believed; and slight variations in diet seem to have very profound in-

fluences on sensitization. For example, carrots grown in one place, without artificial fertilizer, contain 19 parts per million of iodine, while those grown with artificial fertilizer contain 2,100 parts per million. So one can see what variations of metalloids and metals the same food is capable of undergoing.

Regarding what Dr. Waldbott said, naturally we know that the idiosyncrasy of the individual is of primary importance. But in arsphenamine sensitivity there seems to be another factor besides, and that is probably the type of drug. There is a great deal of evidence in favor of the supposition that certain factory lots of arsphenamine suddenly cause series of acute yellow liver atrophies, or dermatitis, or nitritoid crises; this whole series of sensitivities was called by Dr. Harrison, the English investigator, the "X" factor in the drug.

On the question of insecticides in the stables, we have tried to keep the experiments as free from such variables as possible. We have worked in three different guinea pig stables in New York City, and always with practically the same results. So I do not think it could be one such factor that is preventing the New York pigs from getting sensitized.

Dr. Simon and I feel that we have not, as yet, sufficiently tried the transference of food and pigs. The experiments of Dr. Mayer and myself have been going on for over six years. We have used over a thousand guinea pigs in variations of these experiments. Between Breslau and New York the work is very complicated and difficult to do. But Dr. Simon and I hope that we can continue this way, between Boston and New York, and find out something more definite, now that we have two places so close together.

In closing I should like to say that working with arsphenamine is a very clear way to try to find out what extraneous factors influence guinea pig sensitization. One has a constant in the drug, and not a mixture of proteins and unknown substances. One knowns the dosage and the substance one is injecting. One important aspect of these experiments is the variation in apparently exactly similar experimental animals. I have learned, in this manner, to be hesitant in saying that such and such a man's work is no good just because it cannot be checked in another locality. If I had not done the experiments in Breslau, New York, and Zürich myself, with the same technic and with the identical arsphenamine, I should, in New York, have come to the conclusion that the man in Breslau or Zürich was imagining his results.

Dr. Warren T. Vaughan, Richmond.—If I may enter the discussion a little late, Dr. Sulzberger, just one thing occurred to me while you were closing the discussion, which further complicates the situation with regard to the foods.

I have one patient who can eat celery grown in Florida but cannot eat celery grown in Colorado; and another who can eat oranges grown in California but not oranges grown in Florida. Some such factor might apply to the guinea pigs' food.

SKIN HYPERSENSITIVENESS TO EXTRACTS OF TOBACCO LEAF, TOBACCO POLLEN, TOBACCO SEED AND TO OTHER ALLERGENS IN 200 NORMAL SMOKERS*

JOSEPH HARKAVY, M.D.
WITH THE TECHNICAL ASSISTANCE OF MARGARET L. ROSENBERG, M.A.
NEW YORK, N. Y.

IN A preceding study[1] it was reported that extracts of tobacco leaves showed the same allergenic constancy in regard to skin reactions as do the extracts of ragweed, timothy pollen, and horse dander. It was decided therefore: (1) to investigate the incidence of skin sensitivity to tobacco pollen and tobacco seed in thromboangiitis obliterans and control smokers; (2) to determine the allergenic relationship between the tobacco leaf, tobacco pollen and Maryland seed; and (3) to compare the frequency of reactions of the pollen and seed of tobacco with that of ragweed and timothy pollen extracts.

Sixty-nine cases of thromboangiitis and 200 normal controls (smokers) that had been tested with Burley, Coca, Maryland, Virginia and Xanthis tobacco leaf extracts, ragweed, and timothy pollen were also simultaneously tested with the pollen of Burley tobacco mixed with stamens,† pure Maryland tobacco pollen, and Maryland seed.

In a previous communication we described the method of preparation of the tobacco extracts. The tobacco pollen extracts were made according to the Coca technic employed in extracting ragweed and timothy pollen. Buffered saline, however, was used for the extraction of the seed.

The total nitrogen content of the extracts employed in the tests consisted of Burley pollen 0.074 mg. N per c.c., Maryland pollen 0.38 mg. N per c.c., Maryland seed 0.014 mg. N per c.c. Tests were carried out in the same way as those with the tobacco leaf. The results were as follows:

Thirty per cent of the 200 controls reacted to the tobacco pollen and seeds, while 38 per cent reacted to the leaf. In the thromboangiitis group 59 per cent reacted to the pollen and seed in contrast to 78 per cent to the tobacco leaf. The manner in which these groups reacted to the pollen and seeds is indicated in Tables I and II.

It should be noted in Table II that despite the fact that the nitrogen content of pure pollen, such as Maryland (0.38 mg. N per c.c.) was higher than that of the Maryland leaf (0.09 mg. N per c.c.), the num-

*Read before the Twelfth Annual Meeting of the Association for the Study of Allergy, Cleveland, June, 1933.

†It was impossible to separate the pollen from the stamens.

ber of reactions to that pollen as compared with the leaf was lower both in the controls and in the thromboangiitis patients. This may indicate that either tobacco pollen is a poorer antigen or, what is perhaps more likely, lack of actual contact by human beings with the pollen or seed causes but little sensitization to these substances. The reactions with the impure pollen (Burley), the pure Maryland pollen and seeds, although lower on the whole, nevertheless have a tendency to follow the trend of those seen with the tobacco leaf extracts. They may in general be attributed to the biologic and therefore chemical relationship with the tobacco leaf.

TABLE I

SUMMARY OF THE TOTAL PERCENTAGE OF REACTORS AND THE MAXIMUM DEGREE OF REACTIONS IN TERMS OF ONE- AND TWO-PLUS, ETC., IN 200 CONTROLS AND 69 CASES OF THROMBOANGIITIS OBLITERANS TESTED WITH BURLEY AND MARYLAND TOBACCO POLLENS AND MARYLAND SEEDS

	±	+	++	+++	++++	TOTAL
Controls	4	18	7	1	0	30
Thromboangiitis obliterans	9	35	12	1.5	1.5	59

TABLE II

SUMMARY OF THE PERCENTAGE OF SKIN REACTIONS IN NORMAL CONTROL SMOKERS AND THROMBOANGIITIS OBLITERANS TESTED WITH BURLEY AND MARYLAND TOBACCO POLLEN AND TOBACCO SEED EXTRACTS AS COMPARED WITH TOBACCO LEAF, RAGWEED, TIMOTHY, AND HORSE EPITHELIUM

BURLEY LEAF TOBACCO	BURLEY POLLEN	MARYLAND LEAF TOBACCO	MARYLAND POLLEN	MARYLAND SEED	RAGWEED	TIMOTHY	HORSE EP.
			Control Smokers				
0.09 mg. N	mixed with stamens 0.074 mg. N	0.09 mg. N	0.38 mg. N	0.014 mg. N	pollen 0.01 mg. N	pollen 0.01 mg. N	
per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
26	26	32	17	24	24	14	13
			Thromboangiitis Obliterans				
59	49	38	22	46	7	6	Not tested

On closer analysis certain differences are evident in the manner in which the two groups of individuals react. In the controls there is seen a parallelism between the reactions to tobacco leaf, the tobacco pollen, ragweed, timothy, and horse dander which is not present in the thromboangiitis patients. There is no instance recorded in the controls of any exclusive reactions to tobacco pollen or tobacco seed without a simultaneous reaction to other allergens such as tobacco leaf, ragweed, timothy or horse dander. This parallelism may be regarded as an expression of multiple sensitization characteristic of the allergic constitution to which the reacting controls were subject. In

the thromboangiitis cases, however, while the tobacco pollen follows in a general way the tobacco leaf reactions, there is a decided disproportion similar to that seen with the leaf, between the number of reactions to the pollen of tobacco and pollen of ragweed and timothy.

SUMMARY

In 200 controls (normal smokers) no reactions to tobacco pollen or seeds occurred without a concomitant reaction to ragweed or timothy pollen, etc. This can be interpreted as indicating that reactions to tobacco pollen as well as the tobacco leaf in normal smokers may be an expression of a general allergic response which is usually characterized by multiple sensitization.

In patients with thromboangiitis obliterans, tobacco pollen and seed reactions, while lower than those to the leaf closely approximate them and in contrast to the controls (normal smokers) occur six times as often as skin reactions to ragweed or timothy.

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DISCUSSION

DR. MARION B. SULZBERGER, New York City.—Since starting on such experiments in tobacco hypersensitivity, in which Dr. Harkavy, as you know, is one of the pioneers, I have heard many criticisms of the results of the skin tests. The main one is the following: that we were employing our tobacco extracts in concentrations which caused nonspecific skin reactions; in other words, we were using extracts which were acting as primary irritants upon the skin, and that, therefore, the reactions obtained were of no significance in deciding the question as to whether or not the patients were hypersensitive (or had become sensitized) to tobacco.

The main reason those who voiced this criticism gave for their opinion was that the concentration of the tobacco extract was much higher than that which was usually employed in testing hay fevers and asthmas.

I think that in employing a skin test on a type of disease which has not previously been routinely tested, such as is the case in these vascular diseases, and in employing an allergen which has not been standardized for its activity in eliciting skin reactions, one cannot follow methods used with other allergens and in other diseases. One must find the concentration suitable for testing, by means of standardization experiments on diseased persons and on controls. This is what I have tried to do.

We all realize the truth of this statement when testing other conditions. When one uses trichophytin to test a skin for fungus sensitivity, the concentration is entirely different from that which one uses in testing cases of tuberculin sensitivity; and the concentration of pollen extract one uses in a skin test is entirely different from the con-

centration of a food extract employed. The only way to standardize the extracts is to find a series of cases of a disease in which a certain substance has been incriminated as a causative factor, and then to standardize the extract on these patients and on normal controls; and to see whether there is a marked difference in the number of positives elicited by the extract in the cases of the disease which is supposed to be caused by that agent, and the number of positives among the controls. This is just what Dr. Harkavy and I have done with our extracts.

With the concentration of extracts we employed, we found that controls (normal smokers) reacted in 36 per cent only; whereas, thromboangiitis patients gave, as you have seen, markedly high percentages. In my series, the total was 78 per cent positive in thromboangiitis, as compared to 36 per cent in control smokers and 16 per cent in nonsmokers. Whether or not thromboangiitis is due to sensitization to tobacco, is another question. I think we can say that it probably is, for the following reasons: It is difficult to find nonsmokers, but when you do find them and you can get a fairly representative group, one finds, as stated, that nonsmokers give a much smaller percentage of positive reactions to tobacco extracts (in the way we employed them) than smokers do. This you will recognize as one of the characteristics of a specific sensitization. Those with more intimate contact are more likely to become sensitized. A second check on whether a number of positive reactions to an extract is due to sensitization, is to find out whether the incidence of reaction increases with the age group in children. As far as we have gone, it seems that the older the child tested, the more opportunity he has had to have contact with tobacco in the home and so forth, the higher the percentage of positive reactions. Dr. Pascher and I are now completing the work on such a series of children of different ages.

I think that the skin reaction to tobacco will be found to be the manifestations of a specific hypersensitivity to the plant tobacco and not to any of the poisons, nicotine, etc.

As far as the sensitization etiology of thromboangiitis is concerned, I would not have drawn far-reaching conclusions from the skin reactions of my own series of twenty-odd cases, because this number is far too small. But, when added to Dr. Harkavy's series, I thought my findings significant. Our figures coincided almost exactly and showed a markedly higher percentage of skin hypersensitivity to tobacco in thromboangiitis than in any other group.

On one point am I in complete disagreement with Dr. Harkavy. I was not able to demonstrate the presence of atopic reagins with any regularity in these cases of skin sensitiveness to tobacco. I must consider the wheal reactions I obtained in direct tests as evidence of tobacco hypersensitivity, but not of an atopic sensitization, nor of a condition in any way related to asthma or hay fever. I believe that the findings and personal and family history in thromboangiitis cases would tend to substantiate both of my contentions.

DR. GRAFTON TYLER BROWN, Washington, D. C.—I should like to ask whether Dr. Harkavy has tested his series of nonsmokers with the tobacco extracts, and, if so, what percentage he found sensitized.

DR. FRANCIS M. RACKEMANN, Boston.—I should like to ask Dr. Harkavy whether he tested with an extract of tobacco smoke.

DR. HORACE S. BALDWIN, New York City.—I should like to ask Dr. Harkavy and Dr. Sulzberger how much evidence there is for definite absorption of tobacco in the smoking of cigarets.

DR. HARRY S. BERNTON, Washington, D. C.—I should like to ask Dr. Harkavy what his reasons were for not using the cured leaf. After all, it is the cured leaf which is smoked, and not the uncured leaf.

DR. LEON UNGER, Chicago.—I should like to ask Dr. Harkavy whether any scratch tests were made besides the intradermal tests. In my experience there are very few positive scratch tests to tobacco and a fairly large number of positive intracutaneous tests.

DR. HARKAVY.—As far as the extracts of tobacco are concerned, if you test with only one type of tobacco extract and get a negative reaction, you cannot draw any conclusions whatsoever as to the presence or absence of tobacco sensitiveness. You have to test with at least six or eight extracts from different types of tobacco, because each individual tested may and will react to a different extract of tobacco. One person may be sensitive to Turkish tobacco only, another to Burley or Virginia, and so forth. This is substantiated by clinical experience. Some patients will tell you that they can smoke Lucky Strikes and cannot smoke Murads. One man will tell you he can smoke a cigar but not cigarets, another will become sick from smoking a certain grade of pipe tobacco and yet will smoke any number of cigarets with impunity or vice versa.

As far as the statement of Dr. Metzger, that he has not observed any asthmatic individuals in his district with asthma due to tobacco, that may be so, but is readily explained. We know by experience that certain antigens have a peculiar affinity for certain tissues. Phenolphthalein will give a fixed type of eruption in certain areas of the body. Poison ivy invariably affects the skin. One rarely sees ragweed pollen give gastroenteritis. One may see it very occasionally if ragweed is swallowed, but one does see ragweed pollen give hay fever or asthma over and over again.

I believe that tobacco has a peculiar affinity for the vascular system. The fact that tobacco may produce asthma is also true, but to a very minor degree.

There are two men here today, who have observed asthma due to tobacco. One of them is Dr. Cohen of Louisville, Kentucky, who told me yesterday that he was treating two tobacco workers suffering from asthma who develop attacks whenever they return to work. A. Brown reported a case of tobacco asthma many years ago. A physician from Montreal told me just now that an allergist in that city who ministered to a child suffering from asthma discovered that the child got an attack whenever the father smoked his pipe. Otherwise the child was free from it.

As to the incidence of skin sensitiveness to tobacco in nonsmokers, I reported that it is 12 per cent. Dr. Sulzberger found 16 per cent of nonsmokers who gave positive skin reactions.

I have had no large experience with tobacco smoke. I began to study this question two years ago, but some other problems came up which prevented me from continuing. I hope to return to this subject in the near future.

I have a note here, "What evidence of absorption of tobacco in smokers?" There must be a certain amount of absorption of tobacco when you suck on a cigaret all day long. Many of you have known people so sensitive to fish glue that when they simply lick a stamp they would get an angioneurotic edema, or hives of the lips. When a person holds cigarets in his mouth all the time, smoking from 50 to 60 a day, swallowing sputum in which tobacco is dissolved, can there be any question as to absorption?

The best reaction obtained with tobacco extracts was in a man suffering from angina pectoris. He had used tobacco snuff for over thirty years. This finding seems to suggest that there is probably a better chance of sensitization with the induction of positive skin tests when a sensitizing agent is absorbed intact via the respiratory tract than by other routes, such as the gastrointestinal for example. This is substantiated by the general experience with pollen sensitization, which as you know gives the most marked and numerous skin reactions as well as blood reagins, when compared with other allergens.

As far as the scratch test with tobacco is concerned, it is highly unsatisfactory. I have rarely seen skin reactions to tobacco with this method. There must be something in the nature of the antigen that does not lend itself to this method of testing. When you test so many hundreds of patients as I have, by now practically a thousand, and obtain no results with the scratch method worth talking about but do find not only positive skin reactions to tobacco but tobacco reagins as well with the intradermal method, there is ample justification for employing the latter technic.

I want to emphasize one more thing. Reactions in the so-called normal controls which I reported today are far from being nonspecific irritative skin tests. More than half of those who showed positive skin reactions to ragweed, timothy, and tobacco gave either a personal or a family history of allergy.

In a number of asymptomatic patients already investigated who gave positive skin tests we have also detected blood reagins for ragweed, timothy, and tobacco.

If we may regard these 200 normal controls as representing a fair sample of the general population, our observations may mean two things: first, that approximately 38 per cent to 40 per cent of individuals are potentially allergic; second, that specific blood reagins in the asymptomatic individual may indicate the immunologic basis for the clinical concept of "predisposition to disease," in this instance within the allergic framework.

SKIN REACTIONS TO TOBACCO AND OTHER ALLERGENS IN NORMAL MEN AND WOMEN SMOKERS*

JOSEPH HARKAVY, M.D., AND ALFRED ROMANOFF, M.D.
NEW YORK, N. Y.

SKIN hypersensitiveness to tobacco in 103 cases of thromboangiitis obliterans tested intradermally was reported by Harkavy et al. in 1932[1] and 1933.[2] Confirmation of these findings in a total of 24 similar cases was published by Sulzberger in 1933.[3]

It was found that between 78[3] and 86[4] per cent of patients with thromboangiitis reacted to tobacco extracts in contrast to 20 per cent of control smokers and 12 per cent of nonsmokers drawn from a general hospital population. In the selection of controls, proved atopic individuals suffering from hay fever or asthma were excluded. This was done because it was discovered that ragweed sensitive individuals usually reacted to tobacco allergens, whereas only 6 out of 103 cases of thromboangiitis were found sensitive to ragweed. The tobacco reactions in the pollen sensitive individuals were interpreted as due to multiple sensitization characteristic of the atopic state.

In order to elucidate this problem further, it was decided to extend our investigations to an unselected group of 200 normal atopic and nonatopic men and women smokers and to test them not only to tobacco but also to other allergens such as ragweed pollen, timothy pollen, and horse dander. These last were employed because they were found to give a minimum of nonspecific irritative reactions as compared with some foods and inhalants, and second, because subsequent study of blood reagins for these allergens could be more readily pursued in those who reacted positively.

Simultaneous testing of normal smokers with these allergens was of value in establishing the incidence of positive skin reactions to tobacco, in discovering latent ragweed or timothy hypersensitiveness with or without active symptoms, and also served to initiate a more thorough search into the allergic background and family history of allergy, which might ordinarily have been overlooked.

The 200 controls consisted of 100 men and 100 women. The men were porters, cooks, etc., and the women mostly nurses. They were as nearly as possible comparable with our cases of thromboangiitis obliterans in age (twenty-four to sixty-one years) and in amount of tobacco used.

*From the Allergy Division of the Medical Department, Mount Sinai Hospital, New York.

There was, however, one significant difference. The control normals had healthy skins, favorable for testing, in contrast to the patients suffering from thromboangiitis obliterans. The latter usually had dry skins, frequently slightly desquamating because of the impaired circulation brought about by vasospasm which interfered with adequate cutaneous blood supply. The existence of angiospasm has recently been actually demonstrated by Wright and Duryee[5] in their studies of the capillaries in Buerger's disease by means of capillary microscopy. It is therefore possible that plus-minus and one-plus reactions in patients with thromboangiitis obliterans may be the equivalent of a one- or two-plus reaction in the normal controls.

The males were tested by J. H., the females by A. R. and M. L. R. The tobacco allergens were extracts of the cured leaves, prepared according to a modified Coca technic. The difference lay in a more prolonged period of dialysis under toluol (approximately three weeks) so as to remove irritating contaminants.

The final extracts of tobacco used, contained the following quantities of nitrogen: Burley 0.09 mg. N per c.c.; Coca 1:10; Maryland 0.09 mg. N per c.c.; Virginia 0.07 mg. N per c.c. and Xanthis (Turkish tobacco) 0.06 mg. N per c.c. The ragweed, timothy, and horse dander extracts contained 0.01 mg. N per c.c. each. The amount employed in every intradermal injection was between 0.01 and 0.02 c.c. Positive reactions were designated as plus-minus, one-plus, two-plus, etc. A one-plus reaction consisted of a wheal approximately 5 mm. in diameter and an erythema between 1 cm. and 2 cm. A plus-minus reaction was one in which the erythema was negligible but the wheal distinctly larger (about 5 mm.), as compared with a completely negative reaction to diluent or other control allergens.

Of the 200 male and female controls, 124 gave completely negative skin reactions to tobacco and 76 positive. Sixty-seven of the latter reacted in various combinations to tobacco, ragweed, timothy and horse dander, the remaining 9, to tobacco only. (Tables I and II.)

TABLE I

CONTROLS, WITH NEGATIVE SKIN TEST TO TOBACCO	NUMBER OF SMOKERS TESTED		
	MALES	FEMALES	TOTAL
Number of individuals negative to tobacco, but giving a positive personal or family history of allergy and positive skin reactions to timothy, ragweed and horse dander. (Allergies?)	4	19	23
Completely negative to tobacco as well as other test allergens.	58	43	101
Total, negative to tobacco.	62	62	124
It should be noted that of the 23 with positive allergic histories who were negative to tobacco, 7 showed skin hypersensitiveness to ragweed and timothy. (See Fig. 1.)			
The 76 individuals who reacted to tobacco, etc., were distributed as shown in Table II.			

If we were to add to the 76 individuals who reacted to tobacco and other allergens the seven who reacted to ragweed, timothy or horse dander but not to tobacco, we would actually have 83, or 42 per cent, of the 200 controls who showed skin reactions to the various allergens employed in this investigation. These findings corroborate in a large measure the anamnestic studies of Vaughan,[6] who in a survey of 251 individuals in the village of Clover, Virginia, found that 50 per cent of this group actually at one time or another in their lives suffered from what he calls "minor allergy," referable to various shock organs. Rowe[7] in a survey of 400 university students elicited a personal history of probable allergy in 35 per cent.

TABLE II

CONTROLS WITH POSITIVE SKIN TEST TO TOBACCO	NUMBER OF SMOKERS TESTED MALES	FEMALES	TOTAL
Positive skin reactions to tobacco plus ragweed, timothy or horse dander with personal histories of hay fever, sinusitis in fall, bronchitis or migraine, etc. (Allergics.)	18	18	36
Positive skin tests to tobacco plus ragweed, timothy or horse epithelium, in individuals with family history of asthma or hay fever on father's or mother's side, with no personal history of allergy. (Potential allergics.)	0	10	10
Positive skin reactions to tobacco plus ragweed, timothy or horse dander with no history of allergy or asthma. (The preponderance of males over females in this category is due to more reliable histories obtained from the nurses owing to the native character and better intellectual equipment of the women. The men on the other hand were mostly drawn from foreign, unskilled laboring classes.)	15	6	21
Total number of positive reactors to tobacco plus ragweed, timothy or horse dander.	33	34	67
Positive to tobacco only with no history of allergy.	5	4	9
Grand total, positive to tobacco and other allergens.	38	38	76

Detailed analysis of the manner in which the controls reacted, indicated that 17 gave positive skin tests to tobacco, ragweed and horse epithelium; 15 to tobacco, ragweed, timothy and horse epithelium; 13 to tobacco and horse epithelium; 9 to tobacco and ragweed; 7 to tobacco, ragweed and timothy; 3 to tobacco and timothy; 3 to tobacco, timothy and horse epithelium; and 9 reacted to tobacco only.

Sixty-nine cases of thromboangiitis obliterans retested to tobacco, ragweed and timothy extracts, showed an incidence of positive skin tests to tobacco, etc., in 54 (or 78 per cent). Only 6 (or 9 per cent) of these reacted to various combinations of tobacco, ragweed and timothy pollen extracts, as compared to 54 (or 27 per cent) out of 76 normal individuals. One thromboangiitis patient reacted to timothy only and not to tobacco.

In view of the fact that we did not test our thromboangiitis patients with horse epithelium, we felt that for the purpose of comparing the

percentage of tobacco-sensitive thromboangiitis cases with the normals, it would be more conservative if the 13* controls who reacted to tobacco and horse dander only, were placed in the category of exclusive tobacco reactors. This, therefore, makes a total of 22 (or 11 per cent) of tobacco sensitive individuals in the controls as compared with a total of 69 per cent tobacco sensitive only in the thromboangiitis obliterans.

The degree of reaction in terms of one-plus, etc., and the number of reactors in the control and thromboangiitis group to tobacco, ragweed and timothy pollens, are seen in Tables III and IV.

It is obvious from Table IV that there is not only a larger number of thromboangiitis obliterans patients sensitive to tobacco than in the

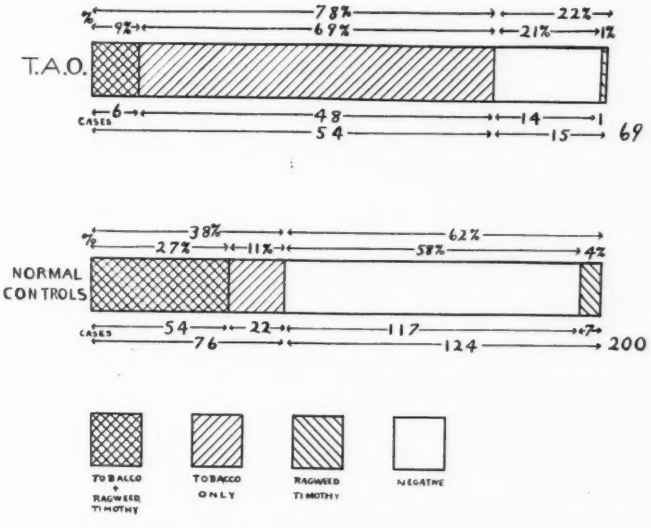

Fig. 1.—Comparison of sensitivity to tobacco between cases of thromboangiitis obliterans and normal controls. T. A. O., thromboangiitis obliterans.

control group, but there are also more patients among them who give three- and four-plus reactions to the tobaccos as compared with the controls.

If we were to disregard the plus-minus and one-plus reactions, we would have 43 per cent of the thromboangiitis patients reacting to tobacco as contrasted with 19 per cent of the controls. This compares favorably with a disease like asthma where demonstrable protein hypersensitiveness occurs in only from 40 to 50 per cent of nonbacterial cases. In view of the concept recognized by allergists that a plus and even plus-minus reaction is significant if clinical contact with the reacting allergen elicits symptoms, our plus-minus and plus reactions should be interpreted in the light of the clinical experience

*For purposes of record it must, however, be stated that 7 of the 13 controls who gave skin reactions to tobacco and horse dander were constitutionally allergic by virtue of personal and family history of allergy.

TABLE III

PROTOCOLS OF REACTIONS OF THE 76 CONTROLS WHO REACTED POSITIVELY TO VARIOUS TOBACCOS, RAGWEED, TIMOTHY AND HORSE DANDER

CASE	BURLEY	COCA	MARYLAND	VIRGINIA	XANTHIS	RAGWEED	TIMOTHY	HORSE EP.	FAMILY AND PERSONAL HISTORY OBTAINED AFTER TESTING
					Males—Controls				
1	+		+	+	+		+++		Father has asthma.
6			+		+				
10	++	++	++	++	++	+		±	
11	+			+	+	+		+	
16	++	++	+++	+++	+++	+	++	+	
20			+	++	++			+	
22	+	+	++	++	++			+	
23	++	++	++	++	++	+			Colds in fall.
24	+	+	++	++	++				
25	+	+	+	+	+	+			
27		±	+						
29		±			+	+			Colds in fall and spring.
32				++	+	+++			
34			+	++	++	+		±	Frequent colds.
42	+	++	++	+	++	+		+	
43	++	+	++	++	++	+		±	Colds in Aug. and Sept. Mother has hives.
48	+	+	+	+	+				
50	±	±	+	+	+			±	
53	+	+	+++	+	+	++		+	
55			±	±		++			Sore throats in summer, gastrointestinal disturbances, colds in summer.
57	+	±	+	++	+	++++	+		
58	++	+	+++	+++	+	+		++	
66			+	++	++	+	±		
67	+	+	+	+	+	++		±	
69	++	++	±		+	+		+	Ulcer. Colds in Aug. and Sept.
71	+	+	++	++	+				Father has asthma.
72	+	+	++	++	++	+		+	
76	++	++	++	+	++	+++	+	+	Patient has eczema.
77	+	+	+	+	++	+		+	Colds in Aug. and Sept.
84	+	++	++	+	+		+		Anginal pains.
88	++	++	+	+	+	+++	+	+	
90	++	+	++	++	++	++		+	Patient has hives. Asthma and hay fever on father's side.
91	+	++		+	+	+		++	Patient has migraine from chocolate.
93	+	+	++	++	++	+	++	+	Patient has hay fever; two uncles have hay fever and dermographia; sister has asthma, niece hives.

TABLE III—CONT'D

CASE	BURLEY	COCA	MARYLAND	VIRGINIA	XANTHIIS	RAGWEED	TIMOTHY	HORSE EP.	FAMILY AND PERSONAL HISTORY OBTAINED AFTER TESTING
100			+	+	+	±	±	±	Nephew has asthma and eczema.
106	+	+	+	+	+	+	±	±	Uncle has asthma.
110	±		±	+	+	±		±	Father has asthma.
112	++	+	+	+	+	±	+	+	Father has asthma.
				Females—Controls					
3	+	+	+	+	+	+	+	+	
5	+		+	+	+				
9				++	++				Grandmother has hay fever and asthma.
13	+	+	++	++	++			±	Brother has asthma.
18	+	+	+	+	+	++	±	±	Mother died of cerebral hemorrhage at 49 years.
23	++	+	+	+	+	++	++		
27	+	+	±	+	+	±	+	+	
30	+	+	+	+	+			+	
31	+	+	+	+	+			±	Colds in September and October.
33	+	+	+	++	++			+	
41	+		++	++		+		±	Hay fever in summer. Cousin has hay fever.
43	+	±	+	+	+		±	+	Frequent colds, hives last summer, cousin on mother's side has hay fever.
53			++	++	+	+	+	+	
57	+		+			++++			Grandfather has asthma.
58				+	+	±			
63	+	+	+	+	±	+	+++	+	
68	±		+	+			±		Has rash on face for 2 years. Father has asthma.
69	+	+	+	++	++			+	
72	+		+	++	++	+	++++	+	Has hay fever in May and Sept. Drug sensitive.
75	++	++	+++	++	+++	++	+	++	Frequent sneezing. Vernal catarrh.
78	++	+	++	++	++	++	+++	+	Eczema. Sister has hay fever.
79			+	±				+	Patient has hives.
80			+		+			±	Patient has hives.
82			+		+			±	Eczema, arthritis, nephew has asthma.
83	+		+					+	Has hives, sinusitis.
84		+							
85	+	+	+	+	+		±	+	Hay fever.
86		+	+		+			+	
87		+						++	Sneezes in A.M.
88				++	++		++		
90	++	+++	++++	++	+	++++	+		Had cough all summer.

TABLE III—CONT'D

CASE	BURLEY	COCA	MARYLAND	VIRGINIA	XANTHIS	RAGWEED	TIMOTHY	HORSE EP.	FAMILY AND PERSONAL HISTORY OBTAINED AFTER TESTING
91	++	++	++	++	++	+	++	++	Had angioneurotic edema. Father cardiac disease.
94		±				±		+	Vasomotor rhinitis from orris root. Sister has hay fever.
97		±		+	+		±	+	
102	+		+	+	+	+			Brother has catarrh in summer? Sinusitis. Dyspnea from smoking. Great-uncle has hay fever.
104	+	++	++++		+	++++			Hay fever in summer.
106	±	±	+	±		±			Hives, sensitive to fur dye. Grandmother has asthma and hay fever.

of the rôle of tobacco in thromboangiitis. The additional fact that blood reagins have been demonstrated by us with the serums of individuals who gave one-plus reactions to tobacco justifies us in considering a one-plus reaction of definite significance.

While 38 per cent of the controls reacted to tobacco, in 27 per cent the reactions were simultaneously paralleled by skin hypersensitiveness to ragweed or timothy, thus leaving only 11 per cent sensitive to tobacco alone. In patients with thromboangiitis, skin reactions to tobacco far outnumber those to ragweed and timothy—in the proportion of 76 per cent of the former to 9 per cent of the latter.

It is apparent therefore that of the substances tested, tobacco is the dominating allergen in thromboangiitis obliterans; while in the controls it may be regarded merely as an expression of multiple sensitivity of the allergic individual, unless proved otherwise.

SUMMARY

1. Intradermal tests in 200 unselected men and women smokers with tobacco, ragweed, timothy, and horse epithelium yielded reactions to those allergens in various combinations in 76 (or 38 per cent).

2. While only 9 of this number reacted exclusively to tobacco, 13 others who gave positive skin tests to horse epithelium and tobacco were placed in the category of reactors to tobacco only, for the purpose of comparison with the tobacco sensitive thromboangiitis obliterans patients, not tested to horse epithelium.

TABLE IV

SUMMARY INDICATING THE PERCENTAGE OF REACTORS AND THE MAXIMUM DEGREE OF REACTIONS IN TERMS OF ONE-, TWO-, THREE-, OR FOUR-PLUS IN 200 CONTROLS AND 69 THROMBOANGIITIS OBLITERANS CASES TESTED WITH FIVE DIFFERENT TOBACCOS, RAGWEED, AND TIMOTHY POLLENS*

	TOBACCO LEAF EXTRACTS					TOTAL	RAGWEED					TOTAL	TIMOTHY					TOTAL
	±	+	++	+++	++++		±	+	++	+++	++++		±	+	++	+++	++++	
200 controls																		
Per cent	1	18	16	2	1	38	5	11	4	2	2	24	4	5	2	1	1	13
Thromboangiitis obliterans																		
Per cent	7	28	23	14	6	78	0	4	0	3	0	7	1,5	1,5	3	0	0	6

*It should be noted that an individual was grouped as a one- or two-plus reactor if he reacted a maximum of one- or two-plus to any one of the five tobaccos tested. Thus for example patient "a" who may have given a one-plus reaction to Burley and two-plus to Maryland was classified as a two-plus reactor.

3. This gives a total of 22 (or 11 per cent) of tobacco sensitive controls out of 200, and 54 (or 27 per cent) sensitive to tobacco, ragweed, and timothy, in various combinations.

4. Of 69 cases of thromboangiitis 54 (or 78 per cent) reacted to tobacco. Only 6 (or 9 per cent) of these reacted to ragweed and timothy.

CONCLUSION

The results of the above investigation indicate that tobacco extracts prepared from the cured leaves are comparable in their allergenic constancy with those of ragweed pollen, timothy pollen, or horse dander extracts.

The strikingly larger number of positive skin tests to tobacco only, in cases of thromboangiitis obliterans, 69 per cent as compared with 11 per cent in the controls, would seem to indicate that of the substances tested, tobacco is the dominating reacting allergen in thromboangiitis obliterans.

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INSULIN ALLERGY*

A Review of Recent Literature and Report of a Case

Marion T. Davidson, M.D.
Birmingham, Ala.

IN THE first few years after the development of insulin, reactions of allergic character were not uncommon. Many of these were thought to be caused by sensitization to extraneous protein contaminants, beef, pork, or other protein, depending upon the source from which the product had been obtained.

As the manufacture of insulin has advanced, the extraneous proteins have been more completely removed, and these reactions have been less common. A number of the cases which were reported can merely be said to have been cases of urticaria occurring during insulin treatment since some had had urticaria before taking insulin, and no studies were reported to confirm the suspicion of the part played by the insulin in the production of symptoms.

There have been a number of carefully studied cases, however. Tuft[1] reported two in 1928. One, he concluded, was caused by sensitization to insulin as it reacted to skin tests to crystalline insulin as well as to the commercial product. The other case he concluded was due to the extraneous protein, as it reacted to the commercial insulin but not to the crystalline.

Allan and Scherer[2] in 1932 reported 100 cases which had occurred in the Mayo Clinic in the years 1927 to 1931. They divided their cases into 84 mild local reactions, 12 severe local reactions and 4 constitutional reactions. These authors were unable to assign the part played by insulin itself in the local reactions. They said that some may have been due to the acidity of the solutions, some to the preservative, and others to extraneous protein. The 4 severe cases giving constitutional reactions were all skin tested with various brands of commercial insulin, and two to crystalline insulin to which they also reacted. Some of their cases were benefited by change in the brand of insulin. In many, the insulin treatment could be discontinued, and in one desensitization was tried with temporary benefit.

The local reactions described consist usually of varying degrees of swelling accompanied by redness and itching at the sites of inoculations. Cade, Barral and Roux,[3] however, reported that in one of their three cases the local edema became so extensive as to require incision.

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Only sterile, serous exudate was found. They told of a case in which sterile abscesses formed at the sites of inoculation, 18 of which were incised in fifteen days.

Urticaria is the type of constitutional reaction reported by all authors, with one exception. Williams[4] in 1930 and again in 1933 reported two cases of gastrointestinal allergy (one in each year) due to commercial brands of insulin. Both patients were relieved by changing from one type of insulin to another, as from beef to pork, or vice versa.

In some cases of allergic reaction to insulin it has been possible to discontinue the use of the hormone because of improvement in the diabetic condition. Several authors, however, reported cases in which the allergic response to the insulin was accompanied by resistance to its action and increased severity of the diabetes. Williams' first patient was in coma, unrelieved by enormous doses of insulin of one type, with immediate improvement following change to a different type.[4] Allan and Scherer[5] in a later report told of a case of diabetes resistant to the action of insulin during urticaria. Rudy[6] also reported a similar case.

As regards the frequency of insulin allergy, opinions differ widely. Allan and Scherer[2] showed a definite tendency to an increase. Beginning in 1927, 14 patients out of a total of 428 diabetic individuals, or 3.2 per cent, showed evidences of sensitivity. There was an increase each year until 1930 when 56 patients of a total of 401 diabetic individuals, or 13.8 per cent, showed symptoms. In 1931, however, there was a drop to 11.7 per cent, but here the figures end so that we do not know whether the decline continued or not.

Joslin[7] in the 1928 edition of his book had seen only four cases of protein type reaction and inferred that those were among his earlier cases. Also, he had had only six cases in which asthma and diabetes had been associated. In two of these the allergy disappeared on development of the diabetes.

Most authors report only one, two, or at most three cases, with no percentage figures. In my own case I can give no percentage figures. It was the only case of protein type reaction encountered in an active diabetic practice of ten years by a widely known southern internist. Hallermann[8] in a total of about 541 cases treated in the period from 1924 to 1930 found only two cases with sufficient symptoms to embarrass the insulin treatment. This is less than 0.04 per cent, and he concluded that the occurrence of insulin allergy is so rare as to be of little concern.

For treatment, many patients have needed nothing further than the discontinuance of the insulin and regulation of the diet. Various types of desensitizing procedures have been attempted at times. Karr, Kreidler, Skull and Petty[9] reported successfully treating one patient

with the serum of a rabbit previously sensitized by injections of the patient's serum. Bryce[10] reported a carefully studied case which she desensitized first by daily and then by twice daily injections beginning with minute amounts and increasing until the patient was able to take the needed amounts twice a day.

Kaufmann[11] in a short communication supplementary to Hallermann's[8] described three cases of severe reaction to insulin. Each was relieved by one or two intracutaneous injections of from 4 to 6 units. His patients were enabled to continue the insulin treatment following this procedure.

Other authors reporting cases of urticaria occurring during insulin treatment are Hajek,[12] Blaisdell,[13] and Grishaw[14] each of whom reported one case in 1931, and Grishaw concluded that the sensitization in his case was to the extraneous protein and not to the insulin.

Cases reported prior to 1927 have not been included in this study because few of them received any allergic study and also because the commercial insulins since that time have seemed to have less extraneous proteins than the earlier specimens.

Several investigators have studied the nature of insulin in the light of its allergic response. It has been stated that insulin is not a pure hormone but a mixture of substances. Abel[15] maintained there is no ground for this statement. He said, "The best theory that can be offered at present for the different species of animal is this: that while these insulins all have the same composition and crystalline form, they differ in respect to the interior arrangement of their component amino acids, and it is on this, just as is the case with various proteins from different animals, that the difference in allergic response rests."

Campbell, Gardiner and Scott[16] found allergic response to purified insulin, but in every case it was less than toward the impure commercial product. They concluded that there was probably a sensitization to organ protein as well as to insulin.

Barral and Roux[17] sensitized guinea pigs with purified insulin and were then able to get shock reactions following second doses. They were not able to get shock reactions from injections of pancreas extracts in pigs sensitized to purified insulin or vice versa, and so they concluded that insulin is a separate antigenic substance.

My own case was Mrs. R. W., aged sixty-two years, seen May 2, 1932, with severe urticaria of four days' duration. There was no allergy in her family history, and she had had no previous allergic manifestations. Diabetes was first diagnosed four years previously, when several doses of insulin were given and diet was prescribed. Since that time she had not taken any insulin and had followed the diet very little if at all.

She entered the hospital April 13 for treatment for diabetes, and diet and insulin were started. The course and laboratory findings are shown in Table I. The insulin was rapidly increased, but in spite of this and rigid diet the urine had not been sugar free a whole day up to the appearance of the urticaria. There was no acidosis at any time.

TABLE I

PROTOCOL OF CASE REPORT

DATE	B. S. MG.: 100 C.C.	U. S.	AC.	DIET	INSULIN DOSAGE	REACTION	REMARKS
4/13/32	333	4 plus	None	P. 20 C. 20 F. 20	None	None	Wt. 153 lb.
4/14/32	250	4 plus	None	Same	None	None	
4/15/32	200	2 plus	None	P. 20 C. 30 P. 20	U. 5 (L) t. i. d.	None	
4/16/32		4 plus		Same	U. 8 t. i. d.	None	
4/18/32	250			Same	U. 15 t. i. d.	None	
4/23/32	133	2 plus	None	P. 20 C. 40 F. 20	U. 18 t. i. d.	None	Wt. 153 lb.
4/28/32		Same	None	Same	Same	Severe rash	Adrenalin Two doses
4/29/32	133	Same	None	Same	U. 9 Tid.	Severe rash	Same Wt. 152 lb.
4/30/32		Same	None	Same	U. 12 Once	Same	Ephedrine given
5/ 1/32		Same	None	Orange juice several times	U. 8(S) Once	Same	Same Wt. 155 lb.
5/ 2/32	200	Same	None	Same c 5% dextrose. B. milk.	U. 8. Once	Same	Ephedrine
5/ 3/32	154	Neg.	None	Buttermilk 1 glass every 3 hours	None	Clearing	No treatment
5/ 4/32		Neg.	None	P. 20 C. 20 F. 20	None	Clear	
5/ 7/32	118	Neg.	None	Same	None	None	Disch. from hosp.
5/15/32	142	Neg.	None		None	None	Wt. 152 lb.
5/18/32	118	Neg.	None				Wt. 148 lb.
7/ 9/32	142	Neg.	None		None	None	Wt. 150 lb.

B. S. Mg.: 100 c.c. = Mg. of blood sugar per 100 c.c.
U. S. = Urine sugar.
AC. = Urindiacetic acid to sol. of ferric chloride.
U. = Units of Insulin.

There were no allergic manifestations either local or constitutional until April 28, the fifteenth day of the insulin treatment, when the rash appeared on the body about an hour after the morning dose. It spread rapidly and soon covered almost the entire skin area, thickly peppering it with large wheals. The insulin was suspected, and the dose diminished without benefit, and May 1, the day before I saw her, the brand had been changed and a dose of Squibb's insulin had been given instead of the Lilly preparation which had been used. During this period there are several notes on the hospital chart that the patient was frantic with itching, and adrenalin and ephedrine were given daily.

On May 2 when I saw her in the afternoon she had had a dose of Squibb's insulin and was so thickly peppered with the eruption that it was difficult to find enough clear skin to put on the tests. All the foods which she was having, and all specimens of insulin in the hospital were tested. The foods included beef, pork, lamb, milk, egg, and wheat. The food tests were all uniformly negative, and the insulins just as uniformly positive.

Three days later a specimen of special pork insulin was received from the Lilly Laboratories, and this was tested along with samples of Lilly, Squibb and Mulford insulins and all were uniformly 4-plus positives.

On June 17 a specimen of crystalline insulin was received from Toronto through the kindness of Dr. D. A. Scott. This was made up without preservative. When skin tested by the side of the commercial preparations the crystalline reacted just like the commercial material.

No insulin was given after the first tests, and on the following day

TABLE II

SKIN REACTIONS TO VARIOUS BRANDS OF INSULIN

DATE	FDS.	FDS.	L. 1	S. 1	L. 2	SP. P.	L. 3	M. 1	L. 4	CST.	L. 5	S. 2
5/ 2/32	Neg.		4 pl	3 plus	4 plus							
5/ 3/32		Neg.	4 pl	4 plus	4 plus							
5/ 5/32				4 plus	4 plus	4 plus	4 plus	4 plus				
6/17/32			2 pl	Neg.	2 plus	1 plus			3 plus	2 plus		
7/ 9/32			2 pl	2 plus		1 plus	1 plus			1 plus		
4/10/33					1 plus	Neg.			Neg.			Neg.
3/30/34				Neg.	1 plus	Neg.				Neg.	Neg.	Neg.

Fds., Foods.
L. 1, 2, 3, etc., Lilly's insulins.
S. 1, 2, Squibb's insulins.
M. 1, Mulford's insulin.
Sp. P., Special pork insulin of Lilly.
Cst., Crystalline insulin from Univ. of Toronto.

the eruption was subsiding rapidly, and no adrenalin or ephedrine was needed after the day the last dose of insulin was given.

From the point of view of the diabetes the condition was, if anything, somewhat better after discontinuance of the insulin. The urine became sugar free and the blood sugar remained near the normal level. There was no acidosis at any time, and the body weight fluctuated little in spite of the rigorous diet. No edema was noted at any time.

From the allergic viewpoint an interesting phenomenon occurred here. The sensitivity rapidly subsided. By the time the crystalline insulin was received from Dr. Scott's laboratory the reactions were getting smaller and it required larger amounts in the intradermal tests to bring reactions. Also, the reaction to the special pork insulin of Lilly disappeared first, and the crystalline reacted about as long as any. These variations are shown in Table II.

The only treatment required by this patient was the withdrawal of the insulin and a few doses of adrenalin and ephedrine. Desensitiza-

tion was under consideration, and had the diabetic condition made it advisable we intended trying the rapid method as described by Besredka and Gloyne[18] for use in horse serum sensitive cases.

In July, I had the patient take the slow desensitizing procedure, beginning with 1 unit and increasing by 1 unit each dose every three or four days up to 8 units. However, by the time this was started the reactions had almost completely disappeared, and I felt that this procedure had little effect on the outcome. She has since taken insulin whenever she felt like it and when seen on March 30 this year had slipped back into her old lax way of dieting and other treatment but was taking insulin without further symptoms. At this last visit, one specimen of Lilly's insulin gave a slight reaction, but the others were all negative. Passive transfer was tried to her son's arm at this time but with negative results.

CONCLUSIONS

1. The purest insulin at present obtainable shows definite allergenic and antigenic qualities and hence is protein in character.

2. Insulin from animal sources is different from the human variety as it is inconceivable that man would become sensitive to a product of his own body.

3. Sensitization to insulin is comparatively rare.

4. The rarity of this sensitization together with the rarity of the coexistence of diabetes and allergy is sufficiently pronounced to suggest the possibility of a causal relationship.

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DISCUSSION

DR. LOUIS TUFT, Philadelphia.—I was interested in hearing Dr. Davidson mention the effects of the hypersensitiveness upon the dosage administered. Several years ago I had an opportunity to study a similar patient reported by Dr. Karr and Dr. Petty. This patient was a diabetic individual who showed a marked local sensitiveness to the insulin, each injection being followed by a very marked local swelling and induration. This patient was found skin-sensitive by test both to the ordinary insulin and to crystalline insulin, and it was interesting to note that coincident with the onset of the local reaction, increasing doses of the insulin were necessary, and with a constantly increasing blood sugar larger doses became necessary. In spite of this, the patient did not appear to be getting any benefit whatsoever from the insulin we administered.

It was thought that if something were not done coma would ensue. On this particular case it was suggested at that time by Dr. Kolmer that rabbits be immunized with insulin and that the anti-insulin rabbit immune serum be used for desensitization. This was carried out, and the patient subsequently apparently lost her sensitiveness; since after several weeks it was possible to return to an extremely small dosage of probably five to ten units—I have forgotten just which—but at any rate, to a very small dose compared with what it had been necessary to administer during the period of sensitiveness.

DR. HARRY L. HUBER, Chicago.—I had one rather interesting experience with a patient who had a dermatitis at the site of insulin injections. Skin tests by the scratch, intracutaneous and patch methods with beef, pork and sheep proteins and with insulin from different sources failed to show any reaction. Our patients use isopropyl alcohol to sterilize their syringe and needle. This alcohol also failed to give a skin reaction. The alcohol that the patient had been using gave a reaction with typical dermatitis. We then found that the pharmacy had accidentally given this one patient alcohol that had been denatured with formalin. Tests with formalin gave a similar dermatitis. This patient was able to use insulin again when formalin was eliminated.

DR. DAVIDSON.—The crystalline insulin used in this case was supplied me by Dr. D. A. Scott of Toronto and was made up without preservative of any kind. The special pork insulin was supplied directly from the Lilly Laboratories. The other insulins used were the ordinary commercial products obtained from the local druggists in Birmingham.

I listened with interest to the discussion which has gone before, on the question of the possible differences in mechanism between atopy and anaphylaxis. I had intended saying something on that subject in closing this discussion. However, now I will only say that if there is a difference in the two mechanisms, and I believe there is, my case probably belongs in the anaphylaxis group as the atopic sensitivity rarely disappears so quickly or so spontaneously as the sensitivity did in this case.

FURTHER STUDIES ON THE LEUCOPENIC INDEX IN FOOD ALLERGY*

WARREN T. VAUGHAN, M.D.
RICHMOND, VA.

IN A PREVIOUS report I presented my reason for believing that a specific leucopenic response may follow the ingestion of individual foods toward which a person is allergic. Detailed analysis was presented of the study of the first case from the point of view of the leucopenic index determination. In this case the leucopenic response to ingested food corresponded so closely to the patient's specific sensitizations as to appear confirmatory of my hypothesis. Since this first case was studied in August, 1933, we have done over 200 leucopenic indices on 60 allergic patients, in an attempt to evaluate the procedure as a routine diagnostic measure.

The test as we are now applying it is carried on as follows: The patient reports to the office in the morning without breakfast, in the postabsorptive state. Two fasting white blood counts are taken at ten-minute intervals. The patient then eats the food to be tested. Following this, counts are made at fifteen-minute intervals for an hour. A final count is made at the end of an hour and a half. A normal range of fluctuation of 1,000 up or down, from the average of the fasting counts, is allowed as normal. A drop of more than 1,000 is considered positive. A drop of less than 1,000, if it occurs in all or nearly all of the counts, is also considered positive.

In a number of instances we have also carried out serial differential counts, bleeding time, clotting time, and blood pressure determination. The results appeared rather inconstant, and since the time required tended to preclude their use as routine diagnostic measures, attention was centered chiefly on the total leucocyte response.

One hundred and ninety-one leucopenic indices form the basis for this report. In all cases the index was compared with the skin reaction, both scratch and intracutaneous. So far as follow-up was possible, these laboratory findings were compared with the patient's subsequent experience from eating the suspected food.

In 118, or 62 per cent, the leucocyte responses corresponded with the skin reactions. In 87, the skin reaction was negative and there was no digestion leucopenia. In 31 the skin test was positive and the ingestion of the suspected food was followed by digestion leucopenia. In this 62 per cent, therefore, the character of the skin reaction confirmed the leucopenic index.

*Read before the Twelfth Annual Meeting of the Association for the Study of Allergy, Cleveland, June 12, 1934.

In 9 cases the leucocyte response was paradoxical, fluctuating through wide ranges both above and below the fasting mean. These could not be interpreted.

The remaining 64, or 33.5 per cent, of the leucopenic indices disagreed with skin reactions. In 24 the skin test was positive while the index was negative. In 40 the skin test was negative but the leucopenic index was positive.

When the two methods of examination agree, nothing much is to be gained by the application of the leucopenic index. If they disagree, however, and the index gives us positive information which the skin test fails to give, then the leucopenic index would be of distinct value.

We were able to follow up the response to eating the suspected food in 67 instances. In 8 the skin test had been positive but the blood response had been negative. Can a person with negative leucopenic index eat the suspected food even though the skin reaction is positive? Of the 8, 3 found that the ingestion of the specific food caused symptoms, while 5 ate the food without symptoms. From this small series we may infer that if the index is negative, the patient probably can eat the food even though the skin test be positive. This would explain in a measure the so-called "false positive" skin test. However, the results were not 100 per cent.

Twenty-one gave entirely negative skin reactions by both scratch and intracutaneous methods but did manifest positive leucopenic indices. This is the group in which one would hope that the index would be of greatest value: the group with so-called false negative skin reactions in whom we would like very much to have available some other method of examination by which we can find the false negative foods. Of the 21, 10 found that the food caused symptoms, while 11 found that it caused no symptoms. In these 10 instances the only laboratory method by which the offending food could be determined was the leucopenic index. This represents 5.2 per cent of the whole. This is a very small percentage on which to claim value for a new method but the actual percentage is far higher. Five and two-tenths per cent represents the *increase* in our laboratory diagnostic efficiency in food allergy with the use of the leucopenic index along with the cutaneous tests. If we are to compare the two methods, we must also include the 62 per cent in which the leucopenic index corresponded each time with the skin reaction. This gives an actual diagnostic effectiveness for the leucopenic index of 67.2 per cent.

The shortcomings of the cutaneous response to food allergens are well known. Even so, they are the best basis we have so far for diagnostic study. The application of the intracutaneous tests represented an improvement over the original scratch method. An understanding of the biologic relationship of vegetable foods has enabled us to find more causative factors in the individual cases, thereby cut-

ting down the number of foods missed by the occurrence of false negative reactions. The food diary has been still another improvement and often helps us to find the causative agent. The elimination diet does likewise. But even so, we continue to miss some offending foods. A method which will increase our present efficiency by an additional 5 per cent is worth considering.

TABLE I

PARALLEL SKIN REACTIONS AND LEUCOPENIC INDICES IN 182 FOOD TESTS

	WHEAT	MILK	EGG	MISCELLANEOUS	TOTAL
Skin negative, blood negative	22	23	21	21	87
Skin positive, blood positive	13	7	2	9	31
Skin positive, blood negative	5	4	4	11	24
Skin negative, blood positive	9	12	11	8	40
Total	49	46	38	49	182

In an additional 9 instances, the leucocyte response was paradoxical.
Miscellaneous foods included: cheese, chocolate, lamb, beef, pork, squash, tomato, banana, orange, apple, corn, turnip, pea, bean, peanut, Irish potato, peach, chicken, cantaloupe, onion, salmon, rye.

TABLE II

COMPARISON OF ACTUAL EXPERIENCE WITH TESTING RESULTS IN 67 PATIENTS WHO SUBSEQUENTLY ATE FOODS IN QUESTION FOR PERIODS SUFFICIENTLY LONG TO REACH DEFINITE CONCLUSIONS

	FOOD CAUSED SYMPTOMS	WHEAT	MILK	EGG	MISCELLANEOUS	TOTAL
Skin negative, blood negative	Yes	0	1	0	1	2
	No	5	4	10	6	25
Skin positive, blood positive	Yes	3	1	0	1	5
	No	3	2	0	1	6
Skin positive, blood negative	Yes	1	0	0	2	3
	No	1	2	0	2	5
Skin negative, blood positive	Yes	2	3	1	4	10
	No	3	4	4	0	11
Total		18	17	15	17	67

If the cutaneous method of study were not available, practically the same information could be obtained by leucopenic index studies. Of 182 foods studied 30 per cent were positive by skin test and 39 per cent by leucopenic index.

Neither method alone nor both together are entirely reliable, as is brought out in the study of the 67 food instances in which the individual took the opportunity to determine definitely whether the foods under consideration were responsible for symptoms. Twenty-seven times, the food was negative by both methods, yet two of these actually did cause trouble. Eleven times, the food was positive by both methods, but in only five, or approximately one-half, did the food actually cause symptoms. This is in agreement with my previous

observation in a study of a series of patients with migraine, that only one-half of positively reacting foods actually cause trouble.

In this follow-up series, of 19 foods giving positive skin reactions, only 8 actually caused symptoms. Of 32 giving positive leucopenic indices, 15 actually caused symptoms.

Of 48 giving negative skin tests, 12, or 25 per cent, did actually cause trouble. These would be classed as false-negative skin tests. Of 35 giving negative leucopenic indices, 5, or approximately 14 per cent, did cause trouble.

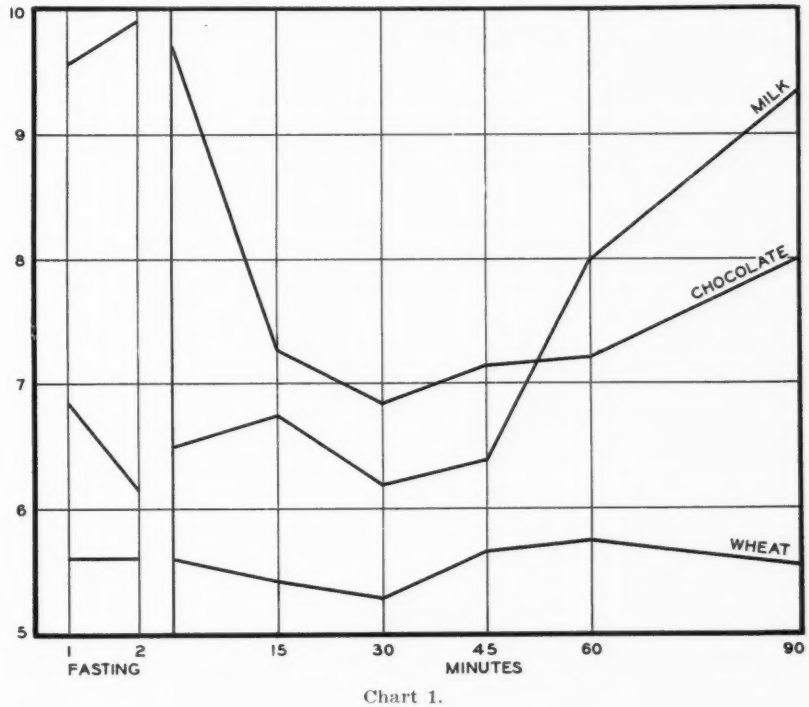

Chart 1.

Repeated indices with the same food do not always correspond. This of course is also true of the cutaneous tests.

It would appear therefore that the leucopenic index method could be used with foods, instead of the skin test method, and with equal efficiency. By this method about the same proportion of false-positive reactions is observed. There is a smaller proportion of false-negative reactions.

The skin test method is much simpler and far less time consuming. The time factor is the great drawback of the leucopenic index method. Its value will lie in its usefulness as an adjunct to the skin test, in the case of doubtful foods, and with those certain foods that experience has shown tend to give false-negative and false-positive reactions.

In a few cases in my experience the leucopenic index has been the only method by which the offending allergen was determined. Without this method in these cases the cause would not have been discovered unless perchance after a long period of trial diets. The first was a case of dermatitis from cantaloupe, the second urticaria from cantaloupe. The third case was of an individual with an extensive dermatitis shown by leucopenic index to be due to wheat. The fourth was of a child with migraine whom we had studied for three months without giving her relief. Her skin test had been entirely negative to milk, and her mother had not collaborated well in elimination diets. When, however, we found a positive leucopenic index to milk, she did cut this completely out of the diet with subsequent relief from the migraine.

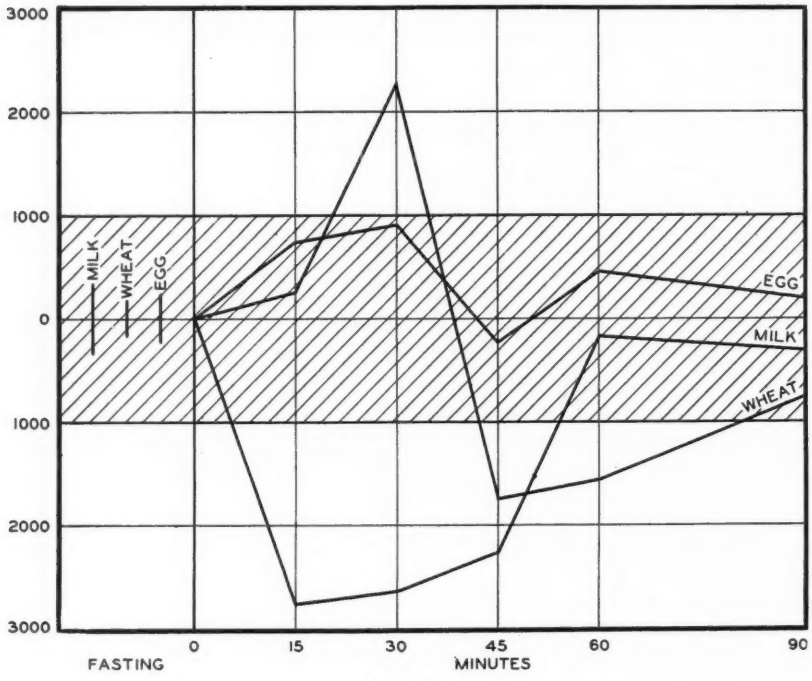

Chart 2.

We have worked out several methods of recording the leucopenic response graphically. Two appeared in the preliminary report of this work. Chart 1 is the routine form used in all cases. It is the simplest and requires no special explanation. This can be varied as in Chart 2, in which the fasting total counts are inserted in the shaded area, their mean being midway between top and bottom. Here the total white count figures must be inserted each time, but the advantage lies in the fact that the variations from the normal range of 2,000 up and down are readily seen.

Chart 3 shows at a glance the variation up and down, and in the lower section also shows the total maximum in both directions.

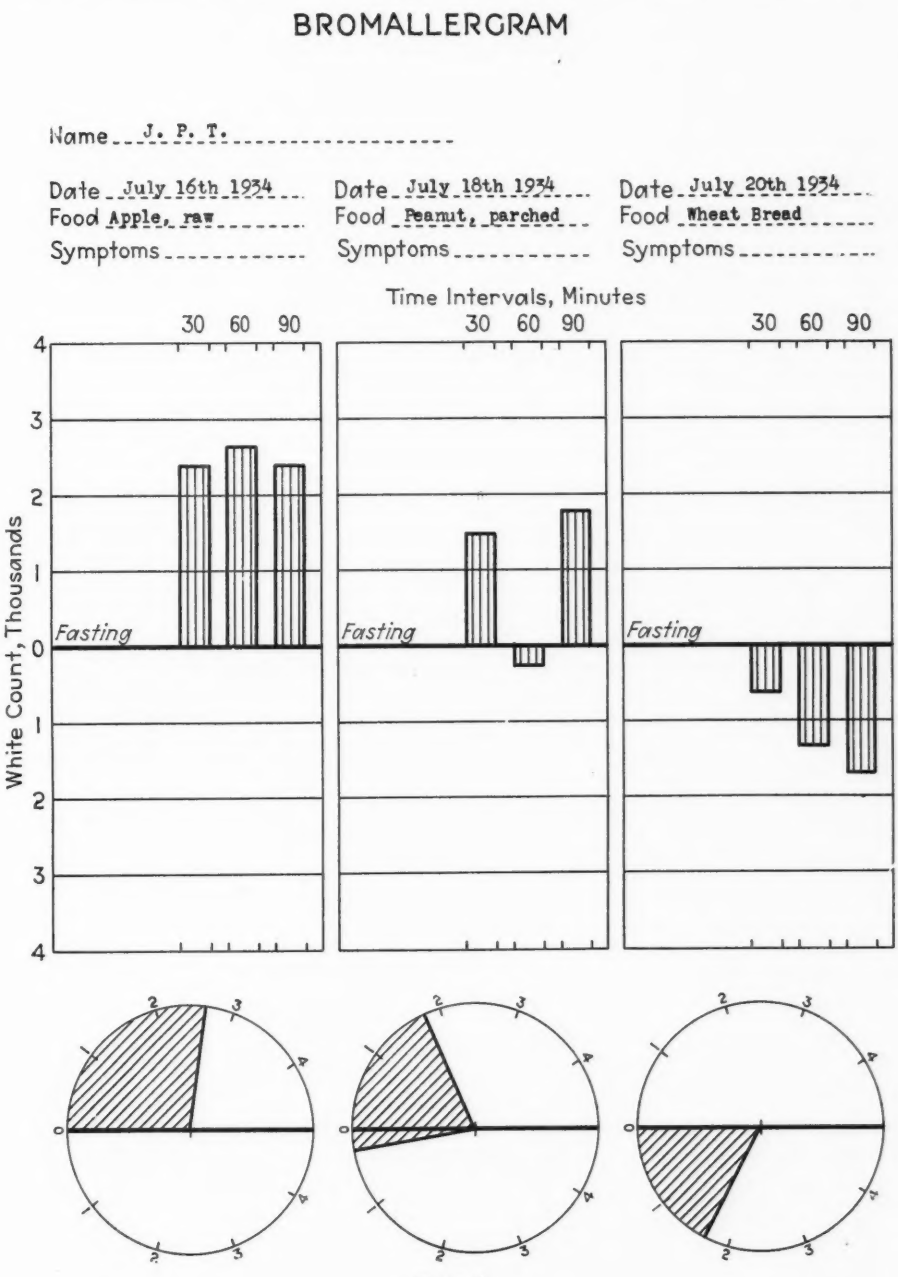

Chart 3.

Chart 4 provides for a quantitative interpretation which might be useful at times. The white count at the end of thirty minutes is charted on the first ordinate; that for sixty minutes on the second; and that

for ninety minutes on the third. Any intervals or any number of intervals may be selected. Only drops of over 1,000 are of signficance. A food showing such a drop on only one of the three sectors would be recorded as one-plus. If the reduction is more than 1,000 on all three occasions, that food would be recorded as three-plus. It will be seen that the graphs of Charts 2, 3, and 4 are derived from the curves of Chart 1, which is our routine work sheet.

SUMMARY

A method is presented which appears to have a reliability equal to that of the familiar cutaneous sensitization test for foods and which could in case of necessity be used instead of the latter, with equal reliability.

The method appears to be specific. Its rationale has been discussed in a previous communication.

Since it is much more laborious than the skin test method, its chief usefulness is as an adjunct to the latter, especially for use with foods concerning which there is still some question and those which experience has shown tend to give false-negative reactions.

When used primarily with just the staple foods, such as wheat, milk and egg, it will increase our diagnostic accuracy by at least 5 per cent. This percentage might be further increased with the use of a wider variety of foods.

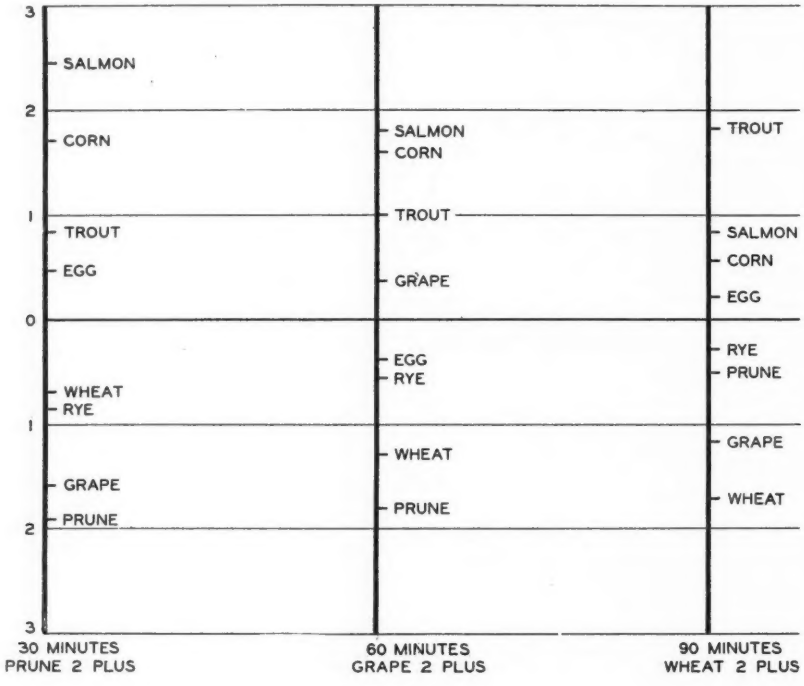

Chart 4.

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DISCUSSION

DR. SAMUEL M. FEINBERG, Chicago.—How long did Dr. Vaughan find leucopenia would last after such experiments, and when did he get his maximum leucopenia?

Another thing that I was not clear about in the paper was, what value it had in the interpretation of possible false-positive skin reactions; in other words, did this leucopenia go along more with the skin reactions which were not responsible for clinical symptoms than with the clinical symptoms?

DR. GRAFTON TYLER BROWN, Washington, D. C.—I should like to ask Dr. Vaughan whether he has made any tests in the inhalant sensitive individuals; and, if this leucopenic drop does not occur in inhalant allergy, would it not be a means of differentiating food from inhalant sensitization?

DR. VAUGHAN.—I had hoped we might be able to find some definite time so that we could make one or two basal counts before and then make the one count, at forty-five minutes or an hour, or some other specified time, later, and know that we are going to find the lowest part of the curve. We have not been able to do it.

Probably the factor of digestion and absorption plays a part. Certain foods are digested and absorbed more rapidly than others. One person's digestive juices are more active at one time than another, so we have found that up to the present it is safest to run a series of counts.

There are so many variables in this thing that it is much safer to run a curve. The circumvention of the false-positive and false-negative skin reactions would be the ideal advantage. We have found, roughly, that we can get correct information by the leucopenic index, in 50 per cent of those skin reactions which are classified as false-positive or false-negative.

I am afraid that we would not be able to use it as a differentiation between inhalant allergy and food allergy because the white count does fall in inhalant allergy also, and, as Dr. Squier brought out yesterday, it may even fall in contact allergy.

I had a very interesting case of a young girl, who was an infectious asthmatic. She came in with a temperature of 102°, due to an acute sinusitis. She had a most severe asthma. Her white count was around 6.000. I gave her an injection of adrenalin. In fifteen minutes her asthma was relieved and her white count had jumped to 13,000. Adrenalin alone in a normal individual will cause some increase in the white count, but I have not seen that much of an increase. My interpretation is that the allergic reaction was keeping the white count down, and the adrenalin released the mechanism, allowing the white count to go up where it ought to be, considering the fever.

FURTHER EXPERIENCES WITH MAXIMUM DOSAGE POLLEN THERAPY*

GRAFTON TYLER BROWN, B.S., M.D., F.A.C.P.
WASHINGTON, D. C.

THREE years ago I presented before this Society a paper on maximum dosage in pollen therapy.[1] It might be profitable at this time to report my further experiences with this method of treating hay fever. Certain refinements in technic have been effected since the preceding report.

In the first place, my extracting fluid has been changed from a 50 per cent glycerine and buffered salt solution, to a slight modification of the Hollister-Stier[2] fluid. Innumerable comparative skin tests have shown that a material increase in the sodium chloride content of the former extracting fluid (from 0.5 per cent to 6 per cent), with a slight decrease in the glycerine (from 50 per cent to 46 per cent), yields more potent pollen extracts, which also have a higher nitrogen content. Patients who have been treated with both types of extracts report greater local reactions, with more itching, following subcutaneous injection of doses of the new extracts. In preceding articles I have stressed the importance of obtaining a satisfactory local reaction from each individual dose, and roughly speaking, the better the reactions, the better the results. Any theoretical objection to the definitely higher salt content can be met when we consider that Clock's[3] original extracting fluid, which is still quite widely used, contained 66⅔ per cent glycerine and 33⅓ per cent saturated salt solution, or about 11 per cent sodium chloride. Pollen extracts with 6 per cent sodium chloride seem to give slightly larger skin test reactions than the 7 per cent sodium chloride extracts advocated by Hollister-Stier. Forty-six per cent glycerine maintains the potency and sterility of the extracts just as satisfactorily as 50 per cent glycerine. As to the stability of glycerine extracts, I might state that extracts prepared from three to five years ago, seem to be just as potent still as freshly prepared extracts. As a buffer apparently is of no advantage in glycerine extracts, the sodium bicarbonate has been omitted. My present extracting fluid, therefore, contains 46 per cent glycerine, 6 per cent sodium chloride, and 48 per cent distilled water. This extracting fluid is not irritating to the tissues, and the individual doses of the pollen extracts prepared with this fluid are injected without further dilution with distilled water or normal saline solution at the time of injection.

*Presented at the Twelfth Annual Meeting of The Association for the Study of Allergy, Cleveland, June 11 and 12, 1934.

To prepare 1,000 c.c. of this fluid, 60 grams of chemically pure sodium chloride are dissolved in 480 c.c. of distilled water, and then 460 c.c. of glycerine are added. It is important to dissolve the sodium chloride in the water before adding the glycerine, as otherwise the salt may precipitate out of the pollen extracts after standing in the refrigerator. If this occurs the salt cannot be redissolved, and shaking merely causes the extract to assume a murky appearance. The extract must then be refiltered before it is used.

My experience with the new dextrose extracting fluid advocated by Unger[4] and his associates, forces me to condemn it. In 1932, when I first examined ragweed pollen extracts prepared with the dextrose menstruum, I noticed that the concentrated extract lacked the rich golden-yellow color possessed by the glycero-saline extracts, and I am always suspicious of pale pollen extracts, that is, the concentrated ones. I found that after the dextrose extract stood in the refrigerator, a dark precipitate settled out, that when shaken, caused the extract to assume a cloudy appearance. I have been informed that this precipitate is the glucoside of the pollen, and that it is allergically inactive. It becomes necessary, therefore, to age the dextrose extracts by keeping them in cold storage for about sixty days, before filtering out this precipitate. Such precipitation has never occurred with glycerine extracts, even when kept for a number of years. No aqueous pollen extracts are as stable as glycerine extracts. As I see it, the only advantage of the dextrose extracts is that, in common with other aqueous extracts, they are easier to draw through small needles, and cause less pain when injected into the arm.

I skin tested quite a number of ragweed sensitive individuals with a mixed ragweed pollen extract in the dextrose solution, and found the freshly prepared extract to be very potent, but apparently not quite so potent as glycerine extracts of a corresponding strength. I have treated only one patient with the dextrose extracts, and she was a young lady with typical seasonal hay fever due to ragweed sensitization. This particular patient was selected for treatment with the dextrose extract because she gave the largest skin reaction to it. I gave her intensive preseasonal treatment, starting May 6 and treating her every four days (25 doses in all) until August 16, at which time she was getting a dose of 60,000 units, or 2 c.c. of a 3 per cent extract. In spite of this treatment, she had severe hay fever, and naturally was discouraged and disappointed with the results. This was my only real failure that season, and I believe that had I given this patient the same amount of treatment with glycerine extracts, she would have had a different story to tell.

Theoretically, the dextrose in the fluid under discussion would seem to offer excellent pabulum for contamination by bacteria or fungi,

which is not entirely offset by the 0.1 per cent cresol added. In support of this opinion, I might state that my last experience with the dextrose-cresol fluid was in the preparation of a concentrated pollen extract for experimental purposes. In spite of the usual care in handling, this extract became contaminated with some yeast organism, which not only ruined the extract, but actually blew the stopper out of the bottle. I have yet to see my first glycerine extract become contaminated. I have observed, however, a definite mold contamination of an alcoholic-saline extract which contained 1.0 per cent merthiolate as a preservative. I have tried many different methods of preparing extracts, but so far have never seen anything to compare with glycerine for maintaining the potency and sterility of the solutions. Hence I feel that I cannot recommend glycerine extracts too highly.

Since 1931, I have been preparing nearly all the pollen extracts in a concentration of 10 per cent, namely, 10 grams of pollen to 100 c.c. of extracting fluid, or 100,000 pollen units per c.c. Recently, I have been experimenting with 15 per cent pollen extracts, and find that they give definitely larger skin test reactions than the corresponding 10 per cent extracts. Up to the present time, however, the 15 per cent pollen extracts have not been used for treatment purposes. As stated in the preceding report,[1] it is desirable to prepare the extracts as concentrated as possible, in order that the very strong doses necessary for consistently perfect results, may be administered without injecting too large quantities of fluid.

Seitz filters have been found preferable to Berkefeld filters, as it is much simpler and less time-consuming to discard the inexpensive germicidal filtering discs than it is to clean the fragile Berkefeld or Mandler candles.

It is an interesting observation that cutaneous tests with the 10 per cent pollen extracts usually give larger reactions than tests with whole dry pollen dissolved on the skin with tenth-normal sodium hydroxide. Testing with various pollen fractions has been almost entirely discontinued, as all the grass and ragweed hay fever patients tested were found to react in varying degrees to most of the fractions isolated from the causative pollens, and it seemed advisable, therefore, to treat with whole pollen extracts.

Patients who have hay fever from two or more unrelated types of pollen, such as grass and plantain, or grass and ragweed, or tree, grass, and ragweed, are treated with mixtures of extracts of the causative pollens. When a patient who is being treated with both grass and ragweed, arrives at grass season without having attained a completely protective dose of grass extract, then the maximum preseasonal dose of grass is repeated at weekly intervals throughout the grass pollinating period, but the ragweed doses are continually increased during this

time. If, on the other hand, a protective dose of grass has been attained by season, then the grass pollen injections are stopped until after hay fever season is over, and the ragweed pollen inoculations are continued alone. In some cases the ragweed doses cannot be increased so rapidly during the grass season, as the combined effect of the injection of ragweed pollen extract with the inhalation of grass pollen from the air, may bring on symptoms of hay fever or asthma.

Treatment for the average hay fever patient can be started with a dose of 5 pollen units, or 0.05 c.c. of a 1:10,000 dilution, which I designate as A strength. Less sensitive patients may be started with 10 pollen units or 0.1 c.c. of A strength. An occasional highly sensitive patient may require for the initial dose as little as 1 pollen unit, or 0.1 c.c. of a 1:100,000 dilution designated as AA strength. Rather than guess at the initial dose, however, it is a safer plan to test the patient cutaneously with various dilutions of the treatment extract, and then start the treatment with 0.1 c.c. of the strongest solution which fails to react any more than the control. Children are given the same initial and subsequent doses as adults. For the sake of clarity, I shall tabulate the various dilutions of the 10 per cent extracts.

AA	or	1:100,000	=	10	pollen units	or	0.01	mg. of pollen per c.c.
A	or	1: 10,000	=	100	pollen units	or	0.1	mg. of pollen per c.c.
B	or	1: 1,000	=	1,000	pollen units	or	1.0	mg. of pollen per c.c.
C	or	1: 100	=	10,000	pollen units	or	10.0	mg. of pollen per c.c.
D	or	1: 10	=	100,000	pollen units	or	100.0	mg. of pollen per c.c.

These dilutions are prepared by taking nine parts of extracting fluid and one part of pollen extract to make the next weaker dilution. The use of the glycerine extracting fluid for preparing the various dilutions is very important, as it maintains the potency and sterility of the dilutions just as it does the concentrated extract, and thus obviates the necessity of preparing fresh dilutions at frequent intervals. Some workers with glycerine pollen extracts make the mistake of using buffered salt solution or normal saline for preparing the dilutions. Dilutions prepared in this manner contain so little glycerine that they are relatively unstable, which greatly increases the likelihood of constitutional reactions when changing to the concentrated extract.

For several reasons, all the treatments are injected subcutaneously, rather than intracutaneously or intramuscularly. In the first place, the relatively large doses that are required, if injected intradermally or too close to the surface of the skin, will tend to form a blister which usually breaks and leaves a scab on the arm for some days. Subcutaneous injections are preferable to intramuscular, because there is less likelihood of accidentally entering a blood vessel, and also they produce more definite local reactions which furnish valuable information in regulating the succeeding doses.

Strange as it may seem, although I am using stronger pollen doses than ever before, constitutional reactions are encountered much more rarely and tend to be of a milder nature. Several explanations may be offered for this interesting observation. One explanation would be that added experience has enabled me to regulate better the individual doses. Another, that improved technic in administering the inoculations has prevented the extract from immediately entering the blood stream, except on rare occasions. Keeping the patients quiet for at least fifteen to thirty minutes following the stronger doses, prevents a number of constitutional reactions, because exercise, especially in hot weather, increases the circulation and causes the pollen extract to be more rapidly absorbed. Other factors that might be mentioned are the use of glycerine extracts, which are more slowly absorbed from the tissues, and the use of the glycerine extracting fluid in preparing the various dilutions as previously referred to. Another important point is the avoidance of giving too strong doses during the hay fever season while the patient is inhaling considerable quantities of pollen from the air.

One or two patients who were getting very large doses of pollen extract, even though there were no constitutional reactions in the usual form, complained of a mental and physical depression following the inoculations, that seemed to be a form of pollen toxemia. When this occurs, the doses are reduced, and if it still persists, the treatments are stopped.

For those patients who have not attained a completely protective dose by their hay fever season, whatever maximum dose is reached just before the pollen season is repeated at weekly intervals throughout the season. If any individual coseasonal dose is followed within twenty-four hours by a definite aggravation of hay fever symptoms, the next dose is reduced a little. At the termination of the pollen season, I start increasing the doses again, and continue the treatments throughout the year until the patient seems to be as completely desensitized as possible.

An important observation is that all patients on reaching strong doses, show a constant and marked reduction in the cutaneous reactions when they are retested. The maximum dose of pollen extract that I now seek to attain is from 100,000 to 200,000 pollen units, namely, from 1 to 2 c.c. of a 10 per cent extract. The mere reaching of a 100,000 or 200,000 unit dose, however, does not in itself insure complete and permanent immunity from hay fever. Rather than the size of the maximum dose, the ultimate goal seems to be the eradication of the skin test reaction, and the more completely this is accomplished, the more satisfactory and permanent are the results. Freeman[5] calls attention to the diminution of the skin test reactions under treatment, with a complete

disappearance if the doses are carried high enough. He reports one hay fever patient whom he gradually worked up to a maximum dose of 100,000 units of grass pollen extract, resulting in a cure which was accompanied by an abolition of the diagnostic skin reactions.

For those patients who have worked up to very strong doses of from 50,000 to 200,000 pollen units before season, I find that it is wise to suspend the treatments entirely during the specific pollinating period, as massive doses during the season will frequently produce hay fever symptoms. This is undoubtedly due to the combined effect of the large amount of pollen injected with that inhaled from the air, as the identical doses which are taken with impunity before season cause trouble when administered during the pollinating period. None of my sufficiently treated hay fever patients had any trouble last year, with the exception of transitory symptoms immediately following massive doses administered during season. These patients had been taking increases in dosage of from 5,000 to 10,000 pollen units or even more at a time, without the slightest trouble, and yet were unable to stand repetition of the preseasonal doses without developing definite symptoms. From this it would appear that the amount of pollen inhaled from the air during season is infinitely greater than has been previously estimated.

Durham[6] has estimated that if a patient were out of doors standing by the pollen slide during the whole twenty-four hours, the maximum amount he would inhale on the worst day we have had in Washington would be about 4,000 granules. This is allowing 20 cubic yards of air for the twenty-four-hour intake, and multiplying this by the average number of pollen granules per cubic yard of air for the period. This total of 4,000 granules inhaled would represent 8 pollen units, so that the maximum contact in Washington would be only 8 units per day.

Patients who have worked up to doses of 50,000 or more pollen units, and are taking increases of 5,000 to 10,000 units at a time, certainly would not be affected by an increment of only 8 units. Furthermore, it is the exceptional patient indeed who has any symptoms from a dose of 8 pollen units injected out of season.

I can think of only three possible explanations for this marked discrepancy. The first and most likely one would be that the estimates as to the amount of pollen inhaled from the air are grossly erroneous. The second explanation would be that 8 units of pollen applied to the nasal mucous membrane is much worse than 8 pollen units injected subcutaneously. The final explanation would be that the pollen inhaled from the air has a cumulative effect in the system.

When the maximum dose of 100,000 to 200,000 pollen units is reached, this dose is repeated at gradually widening intervals. When the positive skin reactions disappear, that is, when "scratch" tests with

whole dry pollen and with D strength extracts are completely negative, the treatments are stopped entirely. In most cases it is a long and somewhat tedious process to attain complete desensitization, although the results are so uniformly satisfactory that they more than justify the time and trouble required. Although the last word has not been written on pollen desensitization, nevertheless this form of treatment with maximum doses seems to be the best method of securing complete and permanent freedom from pollen asthma and hay fever.

CONCLUSIONS

It speaks well for maximum dosage pollen therapy that after three years' added experience with it, I am able to draw practically the same conclusions that were expressed before.

Provided that extracts of the causative pollens are used, the results in the treatment of seasonal hay fever and pollen asthma are directly proportional to the size of the maximum dose attained before season. By gradually working up to large enough preseasonal doses, namely, from 100,000 to 200,000 pollen units, failures are eliminated from hay fever therapy, and perfect results practically assured. To administer such strong doses, it is necessary to give from 1 c.c. to 2 c.c. of 10 per cent pollen extracts. Although these doses are much larger than are being used by other workers in the field of allergy, my extensive use of them indicates that they may be given with perfect safety and comfort, provided sufficient care and judgment are used in regulating the preliminary increases in dosage in the individual patient. Furthermore, the evidence at hand would seem to indicate that the administration of such massive doses leads to complete and permanent desensitization, with a disappearance of positive skin reactions.

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RAPID HYPOSENSITIZATION*

G. L. Waldbott, M.D., and M. S. Ascher, M.D.
Detroit, Mich.

RAPID hyposensitization, the administration of increasing doses of antigen at short intervals, was originally proposed by Besredka[1] for the prevention of anaphylactic shock in animals. Since then, there has been a widespread belief among clinicians that this procedure is both practicable and safe, particularly for the prevention of shock following injections of animal serum. The experience of Tuft[2] and Blankenhorn,[3] in whose cases shock and death occurred, does not bear out this opinion. One of us (G. L. W.) has had occasion to peruse the records of two cases in which death followed the administration of horse serum in spite of the attempt to desensitize according to Besredka's method.

Freeman[4] adopted the method of rapid hyposensitization for the treatment of atopic individuals. His results, however, are not very encouraging, inasmuch as frequent injections of epinephrine were required to counteract reactions.

It has been brought out elsewhere[5] that during the course of preseasonal hay fever treatment shock may occur despite a proper increase in doses and the strictest observation of the proper precautions if the injections are given at too frequent intervals. This, again, is a potent argument against rapid hyposensitization.

In this series of 25 cases of pollen asthma and hay fever, injections were given at the rate of three to six a day. For treatment, the antigen was selected which appeared to be the dominant factor in the patient's condition on the basis of clinical evidence and skin tests. Pollen extracts were employed in all cases. In addition, extracts of cottonseed, wheat, chicken feathers, and autogenous vaccine were added in four cases.

Inasmuch as 23 of the patients were hospitalized and this measure, in itself, is commonly sufficient to improve an allergic condition, no statistical record of the therapeutic results of this method is presented. Moreover, it is generally known that frequently one or two injections of an antigen, to which a patient reacts, are sufficient to induce freedom from symptoms. This paper, therefore, deals with the practicability of using this method rather than with the therapeutic effects accomplished.

In eight cases, generalized reactions occurred after the first few injections, and further increase in the dose of the antigen was, therefore, not attempted. However, the treatment was continued by frequent in-

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jections of smaller doses. In some of these patients injections were given every three hours without increase in dosage.

In 17 cases it was possible to increase rapidly the dose, starting with an average initial dose of 2 units and concluding on the sixth day with a dose of 5,600 units, without encountering any generalized reaction. We feel that this could be accomplished only because of the following two precautions which were taken: First, the initial dose was small enough to produce only little local edema; and second, each increase was cautiously gauged by the appearance of local edema on the site of the preceding injection. When there was a swelling of any marked extent,

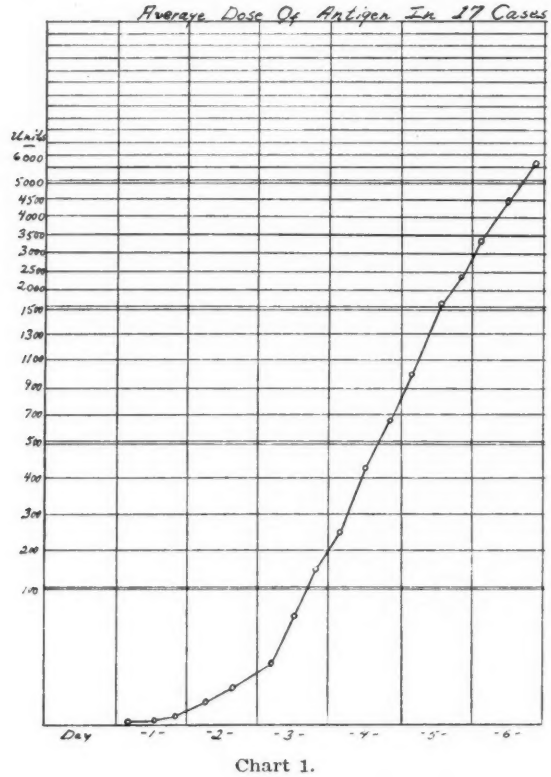

Chart 1.

no attempt was made to force the dose upward. On the whole, no increase above 50 per cent over the previous injection was made. This increase was further reduced and the time interval between injections lengthened if much local reaction was present. No different response was apparent whether the injection was given intradermally or subcutaneously. The intradermal injection, however, because of the more distinct appearance of the wheal, served as a gauge for further treatment to greater advantage.

Eleven generalized reactions were encountered in eight cases. Eight of these reactions were delayed from six to eighteen hours. Three of

the delayed reactions were of marked severity all in asthmatic individuals. They presented aggravation of asthmatic symptoms with a sensation of generalized heat and pruritus, but, according to the patients' statements, definite urticaria seemed not to be present.

Of particular interest is the case of a woman who was so sensitive that a scratch test with a concentrated extract of short ragweed produced a marked generalized reaction within one minute. She stated a similar experience had occurred when she was given an injection of 0.1 c.c. of a 1:1,000,000 dilution of ragweed extract. She had had severe perennial asthma for eighteen years and showed a sensitivity to

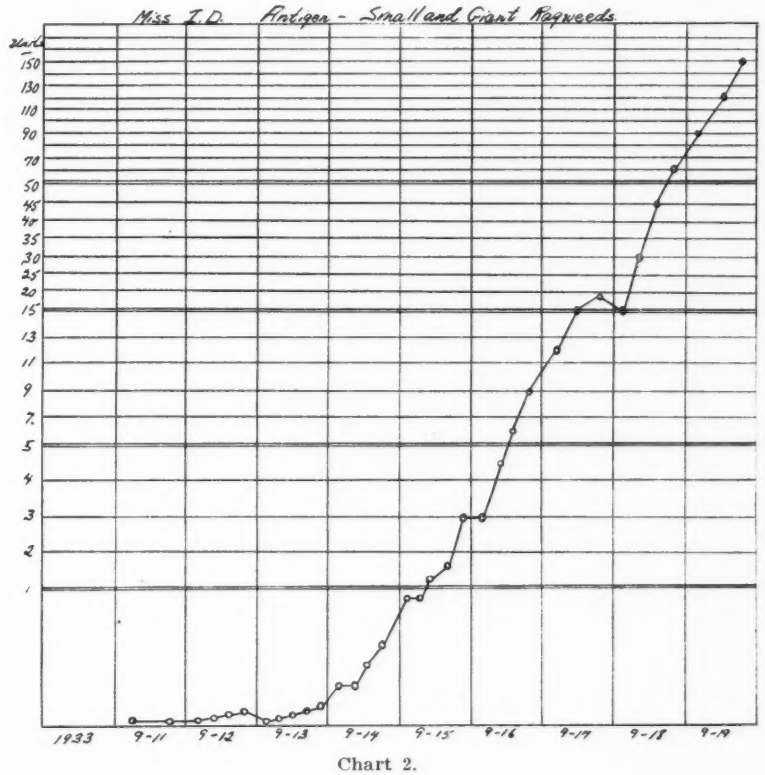

Chart 2.

practically all the common pollens and epidermals and to many foods. She was hospitalized in an extreme status asthmaticus. Being aware of this sensitivity we attempted to produce a local edema without an injection, namely by applying a drop of ragweed extract for a short period upon the scarified skin and washing it off at first after one second, then three seconds, then eight seconds, etc., at intervals of from one-half to two hours. At the end of the second day it was possible to apply the extract to the scarified skin for twenty minutes without a generalized reaction. A marked local edema ensued after each application. On the third day it was possible to inject a dose of $\frac{1}{150}$ of one unit of ragweed

extract without any untoward symptoms. The increase in dosage was carried out as noted in Chart 1. The patient was completely relieved from the asthma for three weeks, one week after her discharge from the hospital.

The following additional observations were made: Treatment with increasing doses appeared to give more lasting results than when the same dose was repeatedly given. It was easier to desensitize with other antigens than with pollens. As to be expected, this treatment can be carried out in the hospital much easier than in the office, probably because of the elimination of other atopens, which are liable to be present in the patient's home surroundings.

The most striking observation is the fact that in some cases there is a tendency toward accumulation of the atopen which is evidenced by the occurrence of reactions, while, for strange reasons, in other cases no such effect was noticed, in spite of the fact that originally marked sensitivity existed. One is reminded of the conditions prevailing in the animal experiment where an antianaphylactic state occurs after a reinjection of a specific antigen. The presupposition of such an antianaphylactic state in man would easily explain the occurrence of late reactions. For, if two injections are given, the accumulative effect of both may not become noticeable for several hours when the state of antianaphylaxis subsides. Whatever the explanation may be, it is well to bear in mind that attacks of asthma which occur within twenty-four hours after an antigen has been given may represent delayed reactions to the preceding treatments.

On the whole, it is felt that it is safer to initiate this treatment without an increase of dosage for a certain period of time and then to increase it rapidly, provided, however, that the dose is cautiously gauged by the appearance of the site of the previous injection.

For practical purposes there may be indications for this method in severe asthmatic individuals where multiple sensitivity exists, particularly if the patient can be hospitalized. Furthermore, in patients living in outlying districts the method may be of use.

SUMMARY

1. By rapidly increasing doses of antigen it is possible to reach a relatively high state of hyposensitization. This method, however, is not void of danger, and a cautious gauging of the doses and of the time intervals between injections is indicated. It should be used only when urgency is necessary.

2. Late reactions occur more commonly than with usual methods of injection of antigen.

3. The treatment can be carried out with greater advantage if several frequent minute initial doses are given before an increase is made. A more rapid increase is then, relatively free of danger.

4. The evidence presented here indicates that it is dangerous to follow the routine for increase in dosage prescribed by Besredka for the prevention of anaphylactic shock.

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602 PROFESSIONAL BLDG.

DISCUSSION ON "DEXTROSE POLLEN EXTRACTS: THERAPEUTIC RESULTS IN 1933" BY LEON UNGER AND MARJORIE B. MOORE (PAGE 561, SEPTEMBER); "FURTHER EXPERIENCES WITH MAXIMUM DOSAGE POLLEN THERAPY" BY GRAFTON TYLER BROWN (PAGE 86); AND "RAPID HYPERSENSITIZATION" BY G. L. WALDBOTT AND M. A. ASCHER (PAGE 93).

DR. ALFRED M. GOLTMAN, Memphis.—I wish to discuss principally Dr. Brown's paper. It makes me feel that there must be a very marked difference in the sensitivity to pollen extracts of his patients and of mine. I appreciate Dr. Brown's position in the field of allergy and truly marvel at his ability to attain such high dosage in his patients. I do not believe, however, that it is possible to attain such a high dosage in my section of the country. What causes the difference I am unable to say, but it might be the food or the climate such as has already been mentioned in regard to differences in the sensitivity of guinea pigs in Boston and New York.

In my experience the maximum dose obtained in some individuals has been 0.5 c.c. of a 2 per cent pollen extract, the average dose has been between 0.15 c.c. and 0.25 c.c. of an extract of the same concentration. Dr. Brown says that he uses 10 per cent pollen extracts and builds his patients up to where they take as much as 1 c.c. of this concentrated solution. With this statement in mind, I should like to ask Dr. Brown a few questions: (1) Is there any saturation point of a pollen extract beyond which no more pollen can be taken up by the solution? (2) Could it be possible that the ability of Dr. Brown's extracting fluid which he has described is after all limited in its absorption power, thereby giving his patients what we might call a chemical protection? I feel confident that practically all solutions have a saturation point and possibly this applies to pollen extracts. (3) In his attempts to eradicate positive skin reactions, does he not endanger the lives of his patients by using such concentrated extracts? (4) It has always been my impression that 3 per cent pollen extracts were obtainable and that after this even if one added more pollen, the extracts were not more concentrated. If this be true, is he really giving more pollen units per cubic centimeter than I should give by using a 3 per cent extract?

I want it distinctly understood that I am not criticizing Dr. Brown in his method of pollen therapy, but am only asking for information.

In discussing Dr. Unger's paper, I should like to know when the general reactions occurred which some of his patients had. Did they occur with high dosage or low dosage? If general reactions occurred by giving doses of a concentrated pollen extract, would these patients have symptoms of hay fever or asthma during the season or were the symptoms greatly ameliorated? If the patients were not relieved, were the symptoms worse in those patients who had general reactions in high dosage? I should also like to know whether or not he has had any comtamination of his dextrose extracts.

Dr. Hal Davison, Atlanta.—I have had no experience with the use of dextrose pollen extracts. We have used glycerin extracts for the reason that we consider them stable and sterile. We have incorporated a small amount of 4 per cent novocaine solution in the material to be injected. This prevents pain even in small children, and they have no fear of the injection. So far we have found no person sensitive to novocaine used in this manner.

I should like to corroborate Dr. Waldbott's experiences. In some cases we have had to begin treatment with 1:1,000,000 solution because a 1:100,000 solution was not tolerated and caused constitutional symptoms. In some of these cases we were not able to increase the maximum dose beyond a solution of 1:10,000.

All of us are more interested in what Dr. Brown said about permanent desensitization than any other point he might discuss, and if there be a safe method by which this can be done, we must not overlook it.

Dr. Albert H. Rowe, Oakland, Calif.—After hearing Dr. Unger's paper last year, I returned to California and immediately prepared some glucose extracts according to his technic. I also prepared comparable extracts with a glycerin-salt solution, and left all of them on the laboratory shelves during the year. They have been tested on susceptible patients every two or three months during the year, and I have found that they produce equally good skin reactions, which have not diminished in size as the months have passed.

This indicates that the glucose extracts are as stable as the glycerin extracts, and that the strength is apparently identical. There has been no contamination, and patients are pleased with the absence of discomfort resulting from the injection of the glucose extracts. In the last few months, I have been extracting all of my pollens according to the recommendations of Dr. Unger. I am very glad again to hear Dr. Unger present the findings that he has accumulated during the past year.

I have also used the rapid method of desensitization with patients who need such treatment preseasonally, according to the former recommendations of Dr. Duke. In January, Freeman published an article in *Lancet* recommending rapid desensitization; I think there is a definite place for it, and, if used with care and the patient is kept for an hour or so after each injection, two or even three injections a day may be given, and the dose rapidly increased, provided due precautions are taken.

It is certainly an important thing to try eventually to eradicate the positive skin reaction, and I am holding it up as the ideal to my pa-

tients. If perennial desensitization is adopted, eradication of the skin reaction can be more frequently attained; however, I want to make this point, that with the disappearance of the skin reaction we cannot be sure that the patient is permanently desensitized; he may go along with negative skin reactions and yet develop definite clinical symptoms that have to be treated.

Dr. George Piness, Los Angeles.—About three years ago the question of pollen solutions and the ability to extract certain percentages of protein from them was thoroughly discussed, and it was agreed at that time that only a certain amount of protein, that is to a certain saturation point, could be extracted. It seemed that the consensus of opinion then as well as now was that no such percentage of 15 per cent to 19 per cent was available by a single extraction and that none of us have been able to extract any such percentage of protein as Dr. Brown has told us today.

As far as stability of glycerin and salt is concerned, we have definitely proved that such solutions are stable, and a study presented and published some ten years ago indicated that salt did as much extracting as any substance one could use, that it was stable, that glycerin added up to 50 per cent would stabilize the solution. Lesser percentages work equally as well.

Most workers have agreed that the reason for pain when these solutions are injected is due to the quantity of solution injected, the location of the injection, and the technic by which it is given. I repeat that the pain is dependent upon the quantity used, the site and technic of injection.

Dextrose solutions have not been used sufficiently by the great mass of workers to bring forth any reports of sufficient value to warrant discarding the buffered salt and glycerin solutions as yet. At the meeting of this society last year I reported results of our observation on solutions—that they remained stable over a period of ten years, kept at both room and refrigerator temperatures. These were buffered salt extractions.

The coseasonal treatment is of great value and is necessary in treating the allergic individuals who come during their pollen season, but I doubt the ability of most men to hold the patients in their offices one hour after each treatment and requiring them to report back three times a day. Fifteen minutes of observation following each injection should be quite sufficient if constitutional reactions are to present themselves. I think that the procedure outlined by Dr. Waldbott is dangerous, impracticable and discouraging to the individual suffering from hay fever.

Reactions are not dependent upon the dose given the patient, since equally severe reactions have occurred from small as well as large doses of pollen antigen. The deaths reported as having been caused by injection of pollen antigen have not been in those individuals where the maximum dosage was given.

Dr. Ben Rappaport, Chicago.—Without being in a position to confirm Dr. Brown's clinical results I am definitely in a position from personal experience to confirm the question of preparation of concentrated pollen solutions. I reported on that last year and have

since repeated the work for my own satisfaction, primarily because of Dr. Piness's various objections and also because that is my method of preparing pollen solution.

The Constitution of this Association, as I understand it, does not demand that one should prepare only a 3 per cent pollen solution. I do not know how that particular concentration originated except from practical work, but I know that if you determine the nitrogen content of a 3 per cent pollen solution it varies between 0.3 and 0.5 mg. N per c.c. If a stronger pollen solution is desired one may use either more pollen or dry the 3 per cent solution and redissolve it in a smaller quantity of water. By this method one may obtain as high as an 18 per cent solution containing 1.8 mg. N per c.c. This seems to be the saturation point of the pollen solution. Clinically, as may be demonstrated by skin tests and by the interchanging of pollen dosage, this concentrated solution is actually six times as potent as a 3 per cent pollen solution.

Dr. Bret Ratner, New York City.—I am a little mystified by the exactness of the chemical extractions that have been described. I should like to know from Dr. Unger and Dr. Brown why they are so careful about getting exactly 46 per cent in our instance and going from 0.5 to 6 per cent sodium chloride in another instance, and what is the reason for using dextrose?

Another question that has been raised and baffles me each year, because the students put it up to me, is: Why do you desensitize people, and how do you bring it about? The only answer I can give them is that when we give small amounts of antigen, the antibodies are drawn out of the cells and are neutralized by the antigen, and this inert combination is excreted through the kidneys.

Now, when large amounts of antigen are given, there is a tendency to form fresh antibodies, and after several days they in turn become anchored to the cells, so it seems to me that giving large amounts creates a vicious cycle, and in turn the patient becomes more sensitized.

I should like to have that discussed because I do not think any of us know the exact rationale for our therapy.

I do think minimum doses of antigen will draw out the antibodies, neutralize them, and result in a better form of desensitization than larger amounts.

Dr. Samuel M. Feinberg, Chicago.—I am merely asking what is a large amount of antigen? Can anybody answer that, whether 3 per cent is large, or 10 per cent, or what is large? Dr. Brown referred to the matter of discrepancy between pollen amounts as injected and as inhaled naturally; i.e., that your patient can have 2 c.c. of a 10 per cent extract, which is a terrific amount, as compared to 4,000 pollen granules by inhalation. He seemed to doubt that pollen counting, as determined by the gravity method, is correct.

I had the same idea several years ago. Mr. Durham did also, and independently we conducted some experiments. My experiment ran something like this: I constructed a box, which could be opened, which had a definite volume, a definite portion of a cubic yard. I covered the bottom of the box with vaseline-coated slides, and then at several periods during the season, I exposed that box on the outside just a matter of three or four minutes and then I closed the box,

sealed it in a paper bag, absolutely air-tight, and let it stand. After the pollen season I counted every slide and, much to my surprise, I discovered the pollen count as determined by such a direct method is just about the same as by the gravity slide method.

DR. FRANCIS M. RACKEMANN, Boston, Mass.—I should like to remark that the trouble with us and with this discussion is, that none of us really knows anything about it. And I will tell you why. Lord Kelvin has said, "When you cannot measure, your knowledge is meager and unsatisfactory." The trouble with us is that we have no measurement of what we do with pollen therapy.

I am perfectly clear in my own mind that skin tests as a measure of sensitiveness are quite unreliable.

I have a friend, a physician, who used to have hay fever and five years ago was treated with ragweed extract. Since then he has had no treatment and he has had no hay fever but his ragweed test is just as good now as it was before, and he still has reagins in his blood. It is obvious that the size of his skin test bears no relation whatever to his clinical symptoms. The point is that we have not any objective measure of the improvement which may result from the treatment we give, and because of that lack it is obvious that we could talk all night.

DR. UNGER.—In regard to Dr. Brown's discussion of the solution that I have been using, let me discuss his points one after another. He condemns the use of the dextrose extracts on six different points. In the first place, it has no rich color, therefore, he is afraid of it; it has not the yellow color which is present in the glycerin extracts. I think all of us who have used dextrose extracts will agree that color has nothing to do with the matter at all. When you see a person get an injection of a dextrose extract and keel over with an anaphylactic shock, as I did two years ago, you know the extract is potent; the 38 reactions we had prove that it is potent.

Dr. Brown also complains that it shakes out on standing. That is true. There is an inactive glucoside which can be filtered out and is of no consequence.

Third, he says you have to keep the material in the ice box 60 days in order to get the precipitate out. That is not true. Two or three weeks is about enough.

Then he says that the absence of pain in giving the dextrose extract is the only advantage he can see in the use of dextrose. I say that that is a great advantage. If you have patients who are taking large doses, especially the dosage that he gives (10 per cent extract), made up with 46 per cent of glycerin, there is considerable pain, especially when he injects 1 or 2 c.c. at a dose. It must be terrific. I would not want to be the patient who received it, and I know that children and nervous people would not like it, either.

The fifth point is that he had one patient and treated the patient with the dextrose extract, and it led to his only failure. I confess to having more failures than that and I do not think he should base his opinion on one failure. He further says that, theoretically, it is a medium for bacteria. We know that it is. That is why we add 0.5 per cent phenol; we have had no contamination. We have given up the Berkefeld filter and now use the Seitz filter; we have had no trouble with the latter.

I should like to ask Dr. Brown, also, when he begins his injections with that dilution which just fails to give a positive test. Does he measure by the scratch method or by the intradermal method? It makes a difference if you use the intradermal, and you will have to start one to a billion in some of the cases.

He mentioned the point that if he gives strong intradermal injections blisters form on the arm. We have had none. You cannot give large doses of glycerosaline extracts intradermally.

We know the good work reported by Drs. Vaughan and Phillips and others on coseasonal treatment. Dextrose extracts can be used coseasonally, preseasonally or perennially.

There is some doubt as to whether glycerin can cause dermatitis and eczema. One allergist thinks that it might cause urticaria. We do not know what other bad effects might follow the giving of 1 c.c. or 2 c.c. of 46 per cent solution of the glycerin extract. It might do some damage to the system over a certain length of time. Dextrose is not harmful.

I should also like to say something about Dr. Waldbott's paper. I have had one occasion to use rapid desensitization. On August 1 a patient let us know she had hay fever, so I gave her an injection every morning and night, and she had a very good result that year.

Dr. Goltman wants to know whether the reactions we had were with high dosages or low dosages. We have had them chiefly with the middle and the high dosages. We have had very few reactions with the 1:10,000 dilutions; more with the 1:1,000.

I do not believe that the symptoms are particularly affected by reactions. I had the idea at one time that a patient with reactions got better clinical results, but I am not so sure about that.

Dr. Davison is using 4 per cent novocaine, and I think that is dangerous. If you use dextrose there is no pain with it and no need for novocaine.

I was glad to hear Dr. Rowe's good reports.

Dr. Piness says there is nothing new in the paper over the paper I gave two years ago. There was nothing new except for the following points: We now use 0.5 per cent phenol instead of 1 per cent cresol. We have used it in 113 cases as reported today and in a lot of others we did not report, and in the previous paper we had only used the extract for two months. Dr. Piness said 5 per cent salt extracts were perfectly stable. I do not think that is correct. As I understand it, the 5 per cent salt extracts deteriorate very rapidly, so that in one season about 50 per cent of the potency is gone. He says pain depends on quantity injected. I think that is true to a certain extent, but if you use dextrose, you will not have much pain.

Dr. Rattner wants to know how we hit on 5 per cent. It happens that 5 per cent dextrose is isotonic with the blood stream and it is stable. It is not an electrolyte. Salt solution is an electrolyte, and Dr. Coca in talking to me this morning said that the salt extracts possibly form an electrolytic solution, causing deterioration.

DR. BROWN.—I wish to thank the gentlemen for their discussion, even though most of it was unfavorable, as I feared it might be. I had hoped, however, that some one would report having adopted my method *in toto*, and could therefore corroborate my results. Most of the questions and objections that have been raised have already been

covered in my preceding paper on the subject, and time does not permit my answering them again now.

Dr. Unger, in his paper, spoke of the difficulty of drawing glycerin pollen extracts through small needles. I use glycerin extracts exclusively, and the smallest needles that can be bought (27 gauge), but have no trouble in drawing them through these needles; although, of course, glycerin extracts do not go through as rapidly as aqueous solutions.

Dr. Goltman questions the administration of such "high" doses. These have been reached through a process of evolution. I did not begin by assuming that I should give hay fever patients what most of you seem to consider veterinary doses. I started in with 1 per cent pollen extracts made in alcoholic saline according to Walker's method, and obtained a certain percentage of perfect results, a larger percentage of improvement, and too large a percentage of flat failures, which I felt was due to insufficient dosage. Then, from year to year, I changed successively to 2 per cent, 3 per cent, 5 per cent, 6 per cent, and finally 10 per cent extracts. My reason for using stronger and stronger solutions was that comparative skin tests showed them to give definitely larger reactions. Skin tests are not infallible, but they are the best guide we have as to the potency of extracts. Let us suppose that you were going to treat an orris-sensitive patient, and had several different orris root extracts. Of these, one gave a definitely larger skin test reaction. You would naturally conclude that this particular extract was more potent than the others, and would use that one for treatment. Well, I feel the same way about pollen extracts, and that is my reason for using such concentrated ones.

I said three years ago and I say again now, that when a patient is treated with extracts of the causative pollen, and yet the result is unsatisfactory, there can be but two reasons for the failure: one is insufficient treatment, and the other is overtreatment. If you give doses that are too strong, you get unpleasant reactions; therefore, if no treatment produces symptoms, you can safely assume that you have not given too much. Under these circumstances, it seems logical to give stronger and stronger treatments until you get the desired result.

I did not say that every one should be given 1 c.c. or 2 c.c. of 10 per cent pollen extract. A large number of my patients never reach that dose. It is a goal, however, that I have never found it necessary to go beyond.

As to the safety of strong doses, I might say that I would rather not treat at all than do anything to jeopardize the health or risk the life of my patients. I have seen no untoward results from maximum dosage therapy, other than the occasional constitutional reactions that we all encounter, and I see even fewer of these than I used to.

We discussed the saturation point of pollen extracts three years ago. I think Mr. Durham is familiar with the extensive series of nitrogen determinations that were done on glycerin pollen extracts of varying strengths (from 1 per cent to 20 per cent). These determinations showed a gradual and regular increase in the amount of nitrogen in the pollen extracts until the 12.5 per cent extract was reached. Comparative skin tests also showed a progressive increase in reactivity of the extracts up to about the same level, closely paralleling the nitrogen determinations. The 10 per cent extracts run better

than 1.0 mg. nitrogen. As these extracts contain more nitrogen and give larger skin reactions than the arbitrary 2 per cent or 3 per cent extracts, they must be stronger.

Lack of time prevents my answering Dr. Davison's questions about the increases in dosage, the intervals, and the premanence of results.

With reference to the hypothetical danger of injecting glycerin, which Dr. Unger mentions, I might say that Clock came out with his original formula in 1917, and that ever since then Lederle has been selling commercial glycerin extracts and reports many thousands of injections. I have never seen dermatitis or hives, or any other unfavorable action from glycerin. The occasional blistering of the skin that I referred to is probably not due to the glycerin, but is apparently caused by the injection of a large bulk of fluid too close to the surface. If 1 c.c. or 2 c.c. of dextrose extract were given intradermally, it would probably blister also.

I use scratch tests for estimating the initial dose.

DR. WALDBOTT.—Dr. Piness spoke about the difficulty of making the patients come into the office for several injections a day. Some of these patients were severe cases and had to be hospitalized. I think they are the ones for whom this treatment is particularly indicated and in whom it is of greatest advantage, provided, however, one takes the proper precautions.

Dr. Piness also brought up the question of constitutional reactions. However, I have elsewhere pointed out that reactions may occur, despite the proper dose, if simultaneous absorption of antigen to which the patient is sensitive takes place, such as the ingestion of food, the inhalation of pollen, etc. However, the most severe reactions are due to accidental intravenous injections, or to the effect of back seepage of extract into a punctured vein.

Since I have made it a practice to withdraw the plunger of the syringe repeatedly before injecting, for evidence of blood, it has happened rather frequently that blood was noticed in the syringe. In this case, automatically, the syringe is withdrawn. An intravenous reaction is exceedingly dangerous.

There is an apparent discrepancy in the results obtained in these cases. In some, astonishingly high doses could be reached within a short time, and in others we had to discontinue the injections. I do not know how this can be explained. In the application of this method, the main problem is to determine when to administer and when to discontinue this treatment, as it is not a procedure which is harmless to the patient.

Department of Reviews and Abstracts

Selected Abstracts

Insulin Allergy. Treatment With Histamine. Collens, W. S., Lerner, G., and Fialka, S.: Am. J. M. Sc. **188:** 528, 1934.

Of 408 insulin-treated diabetic patients, 7.3 per cent showed local insulin hypersensitiveness. The mechanism of this reaction is discussed. It seems to be an antigen-antibody reaction, since local passive transfer gave positive results. Favorable therapeutic results were obtained by insulin desensitization. In view of reported benefit of histamine in the treatment of cases of physical allergy, it was tried in insulin-sensitive cases. In a patient who had been continuously sensitive to all types of commercial insulin for a period of one year, the effect of repeated histamine injections was observed. Injections given 3 times a week with doses beginning with 0.1 mg. and increasing to 1.0 mg. were administered for 13 doses. Insulin reactions diminished. Another course of histamine injections was given, with 0.5 mg. for one dose, then 4 injections of 1.0 mg. each. The local reaction to histamine consisted of severe itching, wheal formation, and erythema. There were also generalized reactions with marked flushing of the face, headache, sweating, sneezing, dizziness, and fall in blood pressure. After the last injection of histamine, insulin was tolerated without reaction, and the patient has since been taking 15 units a day with no local symptoms whatever.

Can Sensitivity to Dinitrophenol Be Determined by Skin Tests? Matzger, E.: J. A. M. A. **103:** 253, 1934.

Tests with dinitrophenol were made in 157 individuals, of whom 117 were patients with hay fever, asthma, and urticaria, and 40 had no allergic complaints. Patch tests, scratch tests, and intradermal tests were made on all. Entirely negative results were obtained in all. Three of these individuals later showed a skin reaction to the therapeutic use of the drug. In one with severe skin reaction, no reagens to dinitrophenol could be obtained by the Prausnitz-Küstner method. These results indicate that skin tests are of no value in predicting skin sensitivity to the therapeutic use of the drug.

Cause of Bakers' Eczema. Zitke, Erna: J. Indust. Hygiene **16**: 218, 1934.

On 149 bakers with eczema, patch tests and subcutaneous tests were made with untreated flour and with flour to which chemicals, as ammonium persulphate, acid calcium phosphate, and potassium bromate, had been added. Forty-one per cent gave positive reactions to untreated flour and 67 per cent to that mixed with chemicals. Of the latter group, 86, or 58 per cent, reacted both subcutaneously to the treated flour and by patch test to ammonium persulphate alone. Only 8 reacted to the chemical alone by patch test and not on subcutaneous injection.

It appears that in these cases the ammonium persulphate is chiefly at fault. Whether this is the sole offender or whether it so affects the wheat albumin to make it more allergenic is open to speculation.

Treatment of Hay-Fever With Amino Acids (Based Upon 29 Treated Cases). Lenormand, J.: Presse. méd. **41**: 1141, 1933.

The treatment of hay fever consisted of a 2 per cent solution of tryptophane, or with a 4 per cent solution of histidin, by daily injections of 0.2 c.c. to 0.4 c.c. under the skin. The key to this treatment was protein therapy. Treatment of hay fever with split products of protein, notably peptone, was recognized. Further split products of albumin, i.e., tryptophane and histidin, were therefore tried. Moreover, peptone injected into the skin becomes reduced through ferments into amino acids. Amino acids and many of their derivatives have a definite action upon the nerves and alter the tonus of the vegetative nervous system. The skin with its contained structures is rich in elements of the vegetative nervous system. By injecting small doses, very marked improvement has been observed. Neither generalized nor local reactions were observed, excepting a mild erythema.

Most of the 29 patients had already been treated by desensitization without improvement. In 14 of these cases there was complete cessation of symptoms, 8 were decidedly improved, and in 5 the relief was less. In many the hay fever diminished in five to six days; in others it took ten to fifteen days; in still others there was improvement with occasional recrudescence, and in a few but partial relief.

The action of amino acids is problematical. They may act by influencing the tonus of the sympathetics, or by protecting the nasal mucosa in making it less permeable to pollens.

Allergic Diseases of the Eye. Urbanek, J.: Wien. med. Wchnschr. **84**: 684, 707, 743, 772, 801, 1934.

Tuberculosis plays a greater rôle in inflammatory conditions of the uvea than is usually appreciated. Likewise, keratoconjunctivitis is

frequently of tuberculous origin despite negative x-ray and other findings. By using the Löwenstein culture technic, a positive blood culture for tubercle bacilli was obtained in a case of keratoconjunctivitis. In such a case, intracutaneous injection of tuberculin leads chiefly to focal reactions in the eye or lung. Of 300 cases of keratoconjunctivitis 92 per cent were found to be allergic. Also tuberculous etiology was found in the cases of chronic uveitis. Tubercle bacilli in the blood and humor of the eye were demonstrated. In all these cases desensitization with "Tebeprotin" led to a positive anergy. Great patience and adequate controls by means of the skin reaction are required in such treatment.

Patch Tests in the Determination of Arsphenamine Sensitization. Robinson, H. M.: Southern M. J. **27**: 845, 1934.

It is generally agreed that the vesicular and exfoliative lesions of arsphenamine dermatitis are due to sensitization to the drug. Patch tests with arsphenamines were made on 165 individuals, of which 22 were nonsyphilitic controls; 54 were syphilitic patients all of whom had received or were receiving arsphenamine treatment without reaction; 18 were syphilitic patients who had developed a previous postarsphenamine jaundice; and 69 had had a previous postarsphenamine dermatitis. The test was made with a 30 per cent solution of arsphenamine in all, and other arsenicals were used in some. Pieces of sterile linen soaked in the various solutions were applied to the skin while wet, and covered with cellophane. Tests were observed twenty-four hours later, and some at forty-eight and seventy-two hours.

Of 22 normal controls, 14 were negative, 5 moderately positive, and 3 strongly positive. In the 51 syphilitic individuals without dermatitis, 3 were strongly positive. Of the 69 with postarsphenamine dermatitis, 54 were negative and 10 were strongly positive. Therefore, although a positive patch test is confirmatory evidence of arsphenamine sensitivity, the test is of no practical value, since it is sometimes positive in normal individuals and often negative in those known to be arsphenamine sensitive. The continuance or interruption of arsphenamine therapy in a patient who has suffered from a postarsphenamine dermatitis cannot be predicated on the results of a patch test.

Tissue Specificity of Allergic Reactions of the Dermis and Epidermis. Sezary, A., and Facquet, J.: Bull. Soc. franç. de dermat. et syph. **42**: 382, 1934.

Tuberculin tests were done by rubbing a concentrated salve containing tuberculin lightly on the skin, or merely by applying it there. In this way the application reaches only the epidermis. To test the effect on the dermis, the skin is rubbed vigorously for thirty seconds with a

tampon soaked in ether, which causes a marked cutaneous reaction. This may be increased if one scrapes the skin with a stone to which the antigen is applied. In cases in which a positive tuberculin reaction occurred, sections of skin were removed for biopsy. Apparently both the epidermis and the dermis may react to tuberculin, but the epidermis is by far the more sensitive.

The Practical Importance of the Regional and Time Difference in Skin Reactivity. Schmidt, W.: Arch. f. Dermat. u. Syph. **168**: 483, 1933.

In a series of experiments it was found that by the intracutaneous method of testing, stronger reactions were obtained on the back than on the arms, and stronger reactions on the underarm than on the overarm, and stronger on the flexor than on the extensor. By the patch method of testing, little or no difference in regional reactivity was noted; or such difference, when noted, appeared to follow no regular distribution.

In general, it was found by either method that a strong positive reaction showed little diminution when the test was repeated within a short space of time.

The patch test is suggested as a more reliable method of testing, since it shows less regional variability in reaction.

Pseudo-ephedrin in Asthma. Bray, G. W., and Witts, L. J.: Lancet **1**: 788, 1934.

At the request of the Therapeutic Trials Committee of the Medical Research Council, comparative clinical tests of the therapeutic value of ephedrine and pseudoephedrine in asthma were carried out. Different species of the Ephedra plant contain different amounts of the alkaloids l-ephedrine and d-pseudoephedrine, and there would be a great saving in cost if they could be used interchangeably. Although it has hitherto been believed that the action of pseudoephedrine is identical with that of ephedrine but weaker, there has been dispute on this point.

Experiments were carried out at the Hospital for Sick Children and at the Research Clinic of Guy's Hospital. To test the effect of the drugs in the prevention of attacks, 20 children, 12 boys and 8 girls, aged from four to nine years, were chosen who (a) had had their asthma for at least two years, (b) had attacks more or less all year round, and (c) had not previously had hospital treatment. The children were divided into four groups of 5 children, and were observed during alternate control periods while taking ephedrine regularly and while taking pseudoephedrine regularly. The dosage employed was ¼ grain morning and evening for a child under seven years, and ½

grain morning and evening for a child over seven years. They were observed for control periods lasting two to four months, and then the same length of time while on each drug.

It was found that pseudoephedrine given by mouth was more efficacious than ephedrine in lessening the frequency of attacks of asthma in childhood. For the relief of paroxysms in adults a standard dose of ½ grain of ephedrine hydrochloride was taken as early as possible in the attack, and repeated at hourly intervals if necessary until 1½ grains had been taken.

Sixty adult patients were so treated, and the side effects of the drugs were observed. It was found that pseudoephedrine was less efficacious than ephedrine in relieving the actual asthmatic paroxysms in adults. It was less toxic than ephedrine; although in large doses it produced the same unpleasant side effects.

It was concluded that pseudoephedrine is worthy of further trial in the treatment of asthma in childhood and in the treatment of adults who are unable to tolerate ephedrine. Neither were so effective as injections of adrenalin in the treatment of the asthmatic attack.

Inheritance of Idiosyncrasies. II. Hay-Fever—The Key Condition of the Allergic Makeup. Hunhart, E.: Deutsche. med. Wchnschr. **30**: 1631, 1934.

There are on record family pedigrees which reveal the inherited tendency of pollen allergy. Through double inheritance an allergic disposition is exhibited most frequently early in life rather than in older people. In these early cases one finds important indications of psychopathic conditions. Hence, in pollen allergy with its early recurrence, easy recognition and frequent association with other allergic manifestations, there is offered an opportunity for the recognition of signs of neuropathies or psychic anomalies in the patient and in his family.

No Evipan in Asthma. Feldweg, P.: München. med. Wchnschr. **80**: 257, 1934.

In a woman thirty-nine years old who had asthma and pulmonary tuberculosis, on whom it was desired to perform a short gynecologic operation, 3 c.c. of evipan were injected over a period of six minutes. This is at a slower rate than usual. The respiration suddenly ceased, and only after an intravenous injection of 10 c.c. of coramine and with artificial respiration did normal breathing return. Inasmuch as evipan is recommended for patients whose respiratory mechanism is at fault, a warning is issued against its use in asthmatic individuals.

Book Review

ALLERGY IN GENERAL PRACTICE. SAMUEL M. FEINBERG. Pp. 339, illustrated, Lea and Febiger, 1934.

Not long ago the author wrote a little book, *Asthma, Hay Fever and Related Disorders, a Guide for Patients.* In it the subject of allergy was related with a simplicity that made it readily understandable to the layman. The present book is designed for the average practitioner, and again the author has a real understanding for his audience. In this instance the complicated subject of allergy is explained in relatively simple terms, but there are ample references at the end of each chapter which serve as a guide to further study.

After short but adequate introductory chapters dealing with the history and more recent developments of allergy, and a consideration of the allergic patient, the remainder of the book is devoted largely to the subjects of asthma and hay fever, with a brief chapter on other allergic disorders. Herein lies the one weakness of the book. Practitioners are likely to be more familiar with asthma and hay fever than with hyperesthetic rhinitis, angioneurotic edema, contact dermatitis, gastrointestinal allergy, etc., to which too little space is devoted. The last 50 pages are given over to case problems which illustrate the application of what appears in the preceding pages.

The book fulfills the purpose for which it is written and should be well received by those whom it serves.